health technology assessment

REPORT

MATERNAL SCREENING FOR FOETAL ABNORMALITY

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EXECUTIVE SUMMARY

INTRODUCTION

Congenital malformations are structural or anatomical defects that are present at birth, resulting from influences acting on the developing embryo in early pregnancy. Some congenital malformations are potentially preventable; however, they remain major causes of early death, hospitalization of infants and young children and significant long-term physical and developmental disabilities. Screening and early detection of Downs Syndrome and other chromosomal anomalies in-utero provides several benefits like the opportunity to inform parents and counseling on the likelihood of delivery of an affected baby. This would allow them subsequently to make an informed decision about whether to continue with the pregnancy or alternatively undergo selective therapeutic abortion to avoid the birth of a disabled baby. The other potential benefits of maternal screening and early diagnosis include preparing the parents psychologically for the delivery of a handicapped baby, enabling doctors to better prepare for the delivery and care of baby, and also avoidance of unnecessary caesarian section in cases with lethal chromosomal abnormality. In addition, maternal screening can also identify foetuses with open neural tube defects.

OBJECTIVE

The objective of this assessment is to determine the effectiveness, safety, cost implications, ethical, legal and social implications of maternal screening for foetal abnormality of the following conditions: Downs Syndrome, Neural Tube Defects and Thalassaemia.

RESULTS

(1) Downs Syndrome

A literature review found that screening using triple serum markers during the second trimester combined with ultrasound to date gestation, increased the detection rate of Downs Syndrome compared to the use of the last menstrual period, so as to avoid performing amniocentesis and chorionic villi sampling (CVS). Ultrasound is used as a secondary tool if serum marker results are positive. It also found that thickened nuchal fold visualized by ultrasound in the second trimester is not a practical screening tool for decisions on offering amniocentesis. However screening using serum markers followed by invasive prenatal diagnosis by amniocentesis or CVS has to be carried out with caution. It was also found that a screening programme is more cost effective than caring for Downs Syndrome children. There are serious ethical and religious issues in screening of maternal serum. It can be concluded that there is sufficient evidence to support the use of triple serum markers combined with ultrasound in second trimester, but there are major ethical and religious issues especially among the Muslim population.

(2) Neural Tube Defects

There is evidence that the use of maternal serum alpha- fetoprotein and ultrasound is effective to detect neural tube defects. However, there is insufficient evidence to support the use of other modalities of screening. It was found that amniocentesis might cause spontaneous abortion. A screening programme showed cost benefits. However, there are major ethical and religious issues especially among the Muslim population.

(3) Thalassaemia

A literature review found that screening tests like MCV/Red cell indices; Hemoglobin A2 estimation, Hb electrophoresis using high performance liquid chromatography or isoelectric focusing; DNA mutation analysis; polymerase chain reaction and other modalities like anti-zeta antibody test are the effective modalities for screening for thalassaemia, However, again there are major ethical and religious issues especially among the Muslim population.

RECOMMENDATION

Due to the ethical and religious issues surrounding prenatal screening, invasive diagnostic procedures and termination of pregnancies, a national programme of routine antenatal maternal serum screening for Downs Syndrome, neural tube defects and Thalassaemia is not recommended. However, screening should be made available to women who request for the test.

1. BACKGROUND

Congenital malformations are structural or anatomical defects that are present at birth, resulting from influences acting on the developing embryo in early pregnancy. Some congenital malformations are potentially preventable; however, they remain major causes of early death, hospitalization of infants and young children and significant long-term physical and developmental disabilities.

There is a lack of local data on birth defects. A study in Hospital Kuala Lumpur in 1996 on 15 535 estimated the incidence of major birth defects to be 0.91%. A subsequent study at University Hospital, Kuala Lumpur in 1970 on 1404 live births found an incidence of 4.7%. A similar study of 14 123 live births in Hospital Alor Setar from 1984-1987 revealed 1.53% birth defects. More recent aggregated national data indicated that 17.9% of stillbirths and neonatal deaths were as a result of lethal congenital malformations. National data on hospital discharges over 1996-1998 indicated that incidence of spina bifida and hydrocephalus ranged from 5.12% (1996), 5.83% (1997) to 5.5.%(1998) of all congenital abnormalities. In 1999, there were 225 cases of neural tube defects out of the 1055 lethal congenital malformations (unpublished data).

2. INTRODUCTION

Currently in Malaysia, routine antenatal care includes the identification of risk factors, health and laboratory screening and counselling. Basic laboratory screening encompasses screening for syphilis, Human Immuno-deficiency Virus, rhesus factor, and Malaria (in endemic areas only). Patients with bad obstetric history such as consecutive miscarriages, perinatal death and abnormal babies are undergo further investigations like Glucose Tolerance Test, LE cell, TORCHES and others based on the related identified risk factors. Ultrasound screening during pregnancy is also carried out at some centers.

Maternal serum screening is used to screen pregnant mothers for foetuses with chromosomal abnormalities like Down's Syndrome and other rare disorders like trisomy 18 and open neural tube defects. Screening and early detection of Down's syndrome and other chromosomal anomalies in-utero provides several benefits. It provides an opportunity to inform parents and provide them with counseling on the likelihood of delivering an affected baby. This would allow them subsequently to make an informed decision about whether to continue with the pregnancy or undergo selective therapeutic abortion to avoid the birth of a disabled baby. Mothers who are screened positive undergo an invasive prenatal diagnostic procedure like amniocentesis or chorionic villus biopsy to confirm or exclude chromosomal abnormality.

The other potential benefits of maternal serum screening and early diagnosis include preparing the parents psychologically for the delivery of their baby who has a handicap, enabling doctors to better prepare for the delivery and care of the baby and avoidance of unnecessary caesarian section in cases with a lethal chromosomal abnormality like trisomy 18. It is also able to avoid late fetal loss in those with a lethal chromosomal

abnormality. In addition, maternal serum screening can also identify fetuses with open neural tube defects.

While currently there is no national maternal serum screening programme in Malaysia, some pregnant mothers obtain screening as part of their obstetric care in the private sector, on a fee-for-service basis. It is also important to note that selective abortion of abnormal fetuses, unless lethal, is not legal in Malaysia.

3. OBJECTIVE

To determine the effectiveness, safety, and cost, ethical, legal and social implications of maternal screening for fetus abnormality of the following conditions: Down's Syndrome, Neural Tube Defect and Thalassemias.

4. METHODOLOGY

Each condition has been assessed separately so that the methodology is elaborated under individual condition.

DOWN'S SYNDROME

1. INTRODUCTION

Down's syndrome (Down syndrome in the United State), also known as Trisomy 21, is a congenital syndrome caused by all or part of trisomy chromosome 21, resulting in mental retardation and other abnormalities. Down's children have a characteristic appearance where the head may be smaller than normal (microcephaly) and abnormally shaped. Other facial features include a flattened nose, protruding tongue, and upward slanting eyes. The inner corner of the eyes may have a rounded fold of skin (epicanthal fold) rather than coming to a point. The hands are short and broad with short fingers and often have a single crease in the palm. Retardation of normal growth and development is typical and most affected children never reach average adult height. They usually have congenital heart defects, with early mortality, often a result of cardiac abnormalities. Gastrointestinal abnormalities such as esophageal atresia (obstruction of the esophagus) and duodenal atresia (obstruction of the duodenum) are also relatively common. Children with Down's syndrome also tend to have a higher than average incidence of acute lymphocytic leukemia (ALL). While there is no proven therapy available for the cognitive deficits, therapies are available for certain specific complications associated with Down's syndrome.

Turner syndrome is a disorder in women usually caused by a missing X chromosome defect that inhibits sexual development and causes infertility. It affects 1 out of 3,000 live births. It is usually sporadic meaning that it is not inherited from a parent. There are many manifestations of this syndrome, but the main features are short stature, webbing of the skin of the neck, absent or retarded development of secondary sexual characteristics,

absence of menstruation, coarctation (narrowing) of the aorta, and abnormalities of the eyes and bones. The condition is either diagnosed at birth because of the associated anomalies, or at puberty when there is absent or delayed menses and delayed development of normal secondary sexual characteristics.

Trisomy 18 or Edwards syndrome is a syndrome associated with the presence of a third (extra) number 18 chromosome. It is a relatively common syndrome affecting approximately 1 out of 8,000 live births and affecting girls more than twice as often as boys. Multiple abnormalities are associated with the presence of an extra number 18 chromosome, many which are not compatible with more than a few months of life. Few infants survive beyond the first year. Common findings include low birth weight, mental retardation, low-set ears, malformed ears, small jaw (microganthia), hand abnormalities, congenital heart disease, hernias, and cryptorchidism. There may be many other abnormalities noted.

The incidence of birth defects is unknown in Malaysia since there is no national registry, but the incidence is estimated to be about 1 in 600 to 700 births. However, the actual incidence of Down's syndrome may be higher than the birth prevalence, as an estimated 48% of affected fetuses are spontaneously aborted at the first trimester and 23% at the second trimester.

The risk of Down's syndrome and other chromosomal abnormalities like trisomy 18 increases substantially with advancing maternal age. Parents carrying chromosome-21 rearrangement and who previously had an affected pregnancy history, independent of advancing age and chromosomal rearrangement are at increased risk of Down's syndrome pregnancies.

The only public health measure available to reduce the incidence of Down's syndrome births, is antenatal screening followed by invasive prenatal diagnosis and selective therapeutic abortion of affected fetuses. Women who are screened positive by serum markers are offered invasive prenatal diagnostic procedures to confirm the chromosomal abnormalities. These procedures differ in their optimal time of performance, invasiveness, risks, accuracy and appropriateness for a given indication. Mid-trimester amniocentesis and chorionic villus sampling are well-established prenatal diagnostic procedures. Other less commonly used procedures are cordocentesis and early amniocentesis.

Maternal serum screening has gained widespread acceptance as a major prenatal screening tool for chromosomal abnormalities. The detection rate depends on the maternal age distribution of the population tested, choice of markers, risk cut-off used and whether ultrasound was used to estimate gestational age.

Patients with positive results for serum marker screening would be subject to amniocentesis or chorionic villi sampling (CVS). CVS is usually done at 10-13 weeks using the transcervical or transabdonimal approach. Culturing differences make the

turnaround time for karyotyping shorter than amniocentesis (7-14 days vs 21), allowing option for earlier termination of pregnancy fetal abnormality when detected.

2. METHODOLOGY

The electronic database Medline, OVID @ Fulltext, International HTA databases and various guidelines were searched for articles or reports published from 1993-2000 using the following keywords: *Down's Syndrome, Maternal Serum Screening, Triple marker screening, Prenatal Screening, Prenatal diagnosis.* In addition, information was obtained from reports from the British Columbia Office of Health Technology Assessment, NHS and U.S. Preventive Services Task Force.

3. RESULTS

3.1 Effectiveness

3.1.1 Benefits of screening

While screening and early detection can theoretically reduce the birth prevalence of Down's syndrome, the actual effect on the birth prevalence will depend on factors like the uptake of screening, uptake of invasive prenatal diagnostic procedures by those women screened positive, and finally, on the uptake of selective therapeutic abortion in the affected pregnancies. The British demonstration project reported a screening uptake rate of 74%, amniocentesis uptake rate of 77% and 79% for termination of pregnancy (Wald NJ et al 1992). The demonstration project in the US showed almost similar results with amniocentesis uptake rate of 79% and 75% for termination of pregnancy (Haddow JE et al 1992).

In some countries, amniocentesis and chorionic villus sampling (CVS) are routinely offered to women aged 35 years or older, who are at exponentially increased risk of giving birth to a baby with Down's syndrome or other trisomy. Performing maternal serum screening in this group and offering amniocentesis to those with a post-screen risk of at least 1:200 can avoid 75% of amniocentesis but at the expense of missing 11% of cases (Haddow JE et al 1994; Wenstrom KD et al, 1995).

3.1.2. Serum markers

Low alpha foeto protein (AFP) levels were initially noted to be associated with Down's syndrome (Merkatz IR et al, 1984). Subsequently other workers noted that pregnancies with a fetus with Down's syndrome have approximately twice the level of chorionic gonadotropin (hCG) of non-affected pregnancies between 15-18 weeks gestation (Borgart MH et al, 1987). Later, low levels of unconjugated estriol (uE_3) were also found to be associated with Down's syndrome (Wald NJ et al, 1988). Subsequently, free beta subunit of hCG has been preferred over total hCG for antenatal screening for Down's syndrome, as it gave a better detection rate(Cuckle H et al, 1992)

There is sufficient evidence to support the use of AFP, uE_3, total hCG, free beta-hCG, free alpha-hCG and dimeric inhibin-A as serum markers for Down's syndrome screening between 15 and 22 weeks pregnancy. The serum markers not found to be useful during this period were Cancer Antigen (CA) 125 and Pregnancy Associated Plasma Protein A (PAPP-A), while SP1 (a gene marker) and alpha-inhibin have been found to be of little value (Wald NJ et al, 1998; Macri NJ et al, 1994; Spencer K, 1993). Multiple marker screening is able to detect 29% of Turner syndrome (Wenstrom KD et al, 1994). AFP is slightly reduced, and UE3 markedly reduced in both hydropic & non-hydropic cases. Those with hydropic pregnancies had elevated hCG levels in contrast to low hCG levels in non-hydropic pregnancies (Saller DN et al, 1992)

A screening programme based on maternal age using a combination of three biochemical markers - AFP, hCG, and uE_3 was proposed in 1998 (Wald NJ et. al 1988). Factors that affect serum marker levels and screening performance include maternal weight, insulin-dependant diabetes, ethnicity and twin pregnancies (Wald NJ, 1998).

3.1.3. First-trimester screening

The serum markers found useful in screening for Down's syndrome between 10-14 weeks gestation are serum PAPP-A and free beta-human hCG (Wald NJ et al, 1998; Krantz DA et al, 1996; Malone FD et al, 2000). Combining maternal age with serum PAPP-A and free beta-hCG can achieve a detection rate of 62% with a 5% false-positive rate, which is comparable to second trimester screening using maternal age combined with the triple test (Wald NJ et al, 1998). The detection rate is improved when combined with nuchal translucency measurement using ultrasound (Wald NJ et al,1998; Malone FD et al, 2000).

While earlier termination of pregnancy is possible with first trimester screening, 25% of affected fetuses would abort spontaneously over the following few weeks. One study has also shown that first trimester screening will not pick up most of the other chromosomal abnormalities (Crandall BF et al, 1993). A review also suggests there is insufficient evidence to support first trimester screening in preference to second trimester screening (Malone FD et al, 2000).

Cordocentesis is usually done after 18 weeks of gestation in women presenting late. In addition, it has been said that amniocentesis in the first trimester is still considered an experimental technique if carried out at 11-14 weeks. (Stranc LC et al 1997).

3.1.4. Screening at 15-22 weeks of pregnancy

The risk cut-off levels of triple markers (serum alpha fetoprotein, uE_3, hCG) used in screening in the second trimester in various studies ranged from 1: 270 to 1:190. The detection rates for Down's syndrome were between 48% and 83% with false positive rates ranging between 4.1% and 10.4%. The false positive rate was found to be lower when ultrasound was used to date gestation (Wald NJ et al, 1992,1988; Haddow JE et al, 1992; Burton BK et al, 1993; Goodburn SF et al, 1994; Philip OP et al, 1992; Benn PA et al, 1995; Kellner LH et al, 1995; Kellner LH et al, 1995; Rose NC et al, 1994)

3.1.5. Screening in women aged 35 years or older

In a study with a sample size of 5 385, it was found that 89% of Down's syndrome could have been identified if amniocentesis was performed in 25% of women with at least a 1:200 risk of carrying a fetus with Down's syndrome. Thus, the triple marker screening could have avoided 75% of amniocentesis at the expense of missing 11% of cases of Down's syndrome. On the other hand, performing invasive diagnostic tests only in these women could have picked up 47% of fetuses with other trisomies, and smaller percentages of other abnormalities (Haddow JE et al, 1994). Another study yielded similar results, the Down's syndrome screening positive rate being 26.1 %, pick-up rate of 75% while 75% of trisomy 18 was also detected (Wenstrom KD, 1995). A prospective study of 3 896 women found that 85% of foetal Down's syndrome had second trimester risk of more than 1:270 showing that maternal serum alpha-fetoprotein screening is more accurate then age alone in determining risk of Down's syndrome (Rose NC et al, 1994).

3.1.6 Amniocentesis and chorionic villi sampling

Studies show that with CVS there is a slightly weaker correlation between the chromosome results obtained from cultured villus and fetal karyotype than is seen in amniocentesis (Smidt-Jensen 1991; Smidt-Jensen et al, 1992).

3.1.4 The role of ultrasonographic examination

When ultrasound is used to date gestation as compared to the last menstruation period (LMP) dates, the standard deviation of the serum markers is smaller. The detection rate of triple test (AFP, uE_3, hCG) is 69% with a 5% false-positive rate when ultrasound is used compared to 59% when LMP is used to date gestation (Wald NJ et. al 1998; Wald NJ et al 1992; Benn PA et al 1997)

A number of ultrasonographic markers are found to be found associated with Down's syndrome. These can be divided into two categories, the first comprising the major structural abnormalities associated with the condition, like congenital heart defects such as atroventricular canal defect and gastrointestinal defects like duodenal atresia. The second category comprises markers such as increased nuchal skin fold thickness, reduced long bone length, pyelectasis, hyperechogenic bowels and choroids plexus cyst. (Benacerraf BR et al 1985; Bahado S et al 1996; Smith-Bindman R et al 2001; Abuhamad AZ et al 1994; Johson MP et al 1993; Johson MP et al 1995; Owen J et al.1994)

Nuchal skin fold thickness is a discriminatory marker, detecting 38% with 1.3% false-positive rate. There are widely different estimates for both the detection rates and false positive rates. Thickened nuchal fold in the second trimester is useful in distinguishing unaffected fetuses from Down's syndrome. The overall sensitivity of this finding is too low to be used as a practical screening test for Down's syndrome (Wald NJ et al, 1998).

The potential value of ultrasonography in screening for Down's syndrome is its use as a secondary screening test to revise the risk in women screened positive by serum markers, but not for primary screening (Wald NJ et al, 1998; Nyberg DA et al, 1995; Smith-Bindman R et al, 2001, Yagel S et al, 1998, Ginsberg N et al,). The use of

ultrasonographic markers as a basis for deciding whether to offer amniocentesis, results in more fetal losses than cases of Down's syndrome detected, and leads to a decrease in the prenatal detection of fetuses with the condition (Smith-Bindman R et al, 2001).

It can be concluded that there is sufficient evidence to support screening using triple serum markers during the second trimester combined with ultrasound to date gestation, thus increasing the detection rate of Down's syndrome compared to the use of the last menstrual period, so as to avoid performing amniocentesis and CVS. Ultrasound is used as a secondary tool if serum marker results are positive. There is sufficient evidence to conclude that thickened nuchal fold visualized by ultrasound in the second trimester is not a practical screening tool for decisions on offering amniocentesis

3.2 Safety

The adverse effects of screening and early detection include complications arising from invasive prenatal diagnostic procedures performed in women found to have a positive screening test result, complications associated with selective termination of pregnancy in affected fetuses and psychological trauma as the result of a positive screening test.

3.2.1. Safety of amniocentesis

The potential complications of amniocentesis include bleeding, infection, puncture of the fetus, and possibly isosensitisation. It is associated with an added procedure related fetal loss rate of about 0.5-1% on top of the background fetal loss rate (Tabor A et al, 1986; National Registry for Amniocentesis Study Group, 1976). Amniocentesis is also associated with an increased incidence of respiratory distress syndrome and congenital pneumonia (Tabor A et al, 1986). However, in another study it was found that the group that underwent amniocentesis did not differ significantly in physical, neurological, or developmental status at one year of age as compared to the control group (National Registry for Amniocentesis Study Group, 1986).

3.2.2. Safety of Chorionic Villi Sampling

The fetal loss rate for transabdominal CVS is comparable to that of mid-trimester amniocentesis (Smidt-Jensen S et al, 1991, 1992). Transcervical CVS results in a higher fetal loss rate with a relative risk of 1.7 compared with either transabdominal CVS or amniocentesis total loss rates of 10.9% vs. 6.3% and 6.4%, respectively (Smidt-Jensen S et al, 1991, 1992; Rhoads GG et al, 1989). An increased risk of transverse limb reduction anomalies in infants born after CVS has been reported (Firth HV et al, 1991; Burton BK et al, 1992). Current estimates for the overall risk of transverse limb deficiency from CVS range from 0.03% to 0.10% of procedures (US Preventive Service Tasks Force, 1996)

3.2.3. Safety of termination of pregnancy

The potential complications of induced abortion include haemorrhage, sepsis, incomplete abortion and uterine perforation. The overall complication rate was 0.9%, while 0.07% patients required admissions for suspected complications, although there was no mortality (Haikim-Elahi E et al, 1990). The mortality of legally induced abortion is

found to be ten times lower than the risk of pregnancy related deaths, that is, 0.4/100,000 procedures compared with 9.1/100,000 live births (Lawson HW et al, 1994; American Medical Association 1992). However, abortions during the second trimester are associated with a higher complication rate including a higher mortality rate (Lawson HW et al, 1994).

3.2.4. Safety of Screening

Screening can cause adverse psychological effects on parents. A positive screening result and the wait for the final results may negatively affect their emotional well-being. Parents fear the discovery of an abnormal baby. They worry about the possible complications resulting from subsequent invasive diagnostic procedures, therapeutic abortions and the final negative amniocentesis results (Statham H et al 1993; Santalahti et al 1996). Studies have shown that women with positive screening test results had significantly higher levels of anxiety than those who had negative results, and they also had a negative attitude towards the pregnancy and the baby(Keenan KL et al, 1991; Marteau TM et al, 1992, 1992; Santalahti et al, 1996). Genetic counseling does significantly reduce their anxiety level (Keenan KL et al, 1991).

Concerns have been raised that medical staff are often unclear about the implications of screening tests and how to interpret risk. They also do not recognize women's concerns while awaiting results of the amniocentesis. It is said that the current strategy for implementation of serum screening does not meet the needs of women with positive results (Statham H et al, 1993).

It has also been pointed out that many women who are not aware of the possible drawbacks often see serum screening as a means of reassurance (Roelofsen et al, 1993).

Thus, screening using serum markers followed by invasive prenatal diagnosis by amniocentesis or CVS has to be carried out with caution.

3.3 Cost-Implications

In British Columbia, an appraisal partly derived from primary data of children with Down's syndrome, estimated the excess cost of health care for a person born with Down's syndrome to be C$350,000 (1997 dollar value)(Bassett K et al, 2000). Another estimate of the lifetime economic costs of Down's syndrome put it as $410,000 (US Preventive Services Task Force, 1996). It is difficult to estimate the lifetime economic costs of Down's syndrome in Malaysia due to the lack of the full complement of medical, rehabilitative and social support to disabled persons.

Screening has been concluded to be cost-effective as the excess health-costs of caring for children with Down's syndrome is so high that it exceeds the costs of providing a screening programme (Bassett K et al, 2000; US Preventive Services Task Force, 1996; Wald NJ et al, 1992). However, it is unethical to propose a screening programme solely based on this reason, as a screening programme aims not to save health care costs, but to give parents the chance to avoid the birth of a disabled child (Bassett K et al, 2000, Wald NJ et al, 1992, 1998).

A screening programme has been estimated to cost £1 564 227 for 100,000 births (Wald NJ et al, 1988). Using the same cost analysis and assuming the total births to be 500 000 annually, the total cost of a screening programme in Malaysia would be RM43 016 242 (exchange rate taken as 1£= RM5.50). However, the actual cost will depend on the uptake of screening, amniocentesis and termination of affected pregnancies.

There is sufficient evidence to conclude that a screening programme is more cost effective than caring for Down's syndrome children.

3.4. Legal, Social, Ethical, and Religious Issues

Prenatal screening, counselling and preventive procedures raise many legal, moral, ethical, and religious issues.
With respect to the legal aspect, selective abortion of abnormal fetuses is not legal in Malaysia unless lethal.

Ethical issues include questions like who would benefit from prenatal screening? Is it carried out for parents to allow them to make informed decisions, for physicians to provide optimal care avoid legal liability, for society to minimize the burden of suffering or health care costs, or for the baby to be provided with a 'good start' in life (Stranc LC et al, 1997)?

A central goal of prenatal counselling is 'informed choice'. Supporters of prenatal screening may argue that it provides women and couples 'choice' and thus, some degree of control over their pregnancy and reproduction. However, this 'choice' is affected by many social circumstances – some women may feel pressured into accepting prenatal screening by their doctors who feel that this is in their best interest. Other women may undergo testing at the insistence of their husbands or other family members. Consequently, many women may be going through a screening programme about which they know very little (Bassett K et al, 2000).

Considered from the disability perspective, disability groups argue that prenatal screening, diagnosis and selective termination of pregnancies have a eugenic potential that constitutes a threat to disabled people as it may foster a more negative image of disability and lead to increased discrimination against the disabled (Bassett K et al, 2000). In the United Kingdom, Muslims do not attend genetic counselling and preventive programmes due to their religious and cultural beliefs that they not wish to interfere with natural events (El-Hashemite N, 1997; Salihu HM, 1997). A study by Roberts and colleagues in Birmingham, UK, about the representation of ethnic minorities at genetic clinics, showed that family doctors refer Pakistani families with genetic disorders less frequently to genetic clinics than families from other ethnic groups, since doctors believe that Pakistani families do not wish to attend prevention programmes due to their religious beliefs (El-Hashemite N, 1997). In Cameroon, where Muslims constitute 25-30% of the population, .it has been said that it is difficult to offer comprehensive guidance for many Muslim couples since abortion is forbidden in Islam (Salihu HM, 1997).

The termination of pregnancy remains a contentious issue in many societies. The Muslim juriconsults (Sunni and She'at) agree that if genetic tests proved definitely that a fetus is affected by serious disease that will keep him disabled after birth, then abortion is permissible and lawful provided the termination of pregnancy is carried out before the time of breathing of the soul, that is, 120 days of gestation (El-Hashemite N, 1997). However, there have been concerns raised that while this may be acceptable to literate Muslims, much of the Muslim population depend on the local Imams (Muslim leaders), most of whom are strongly against termination of life, even before breathing of the soul (Salihu HM, 1997).

However, with second trimester serum screening, amniocentesis is offered at between 15-18 weeks of pregnancy. It takes about 10-14 days for reliable karyotyping and up to 3 weeks in total turnaround time. Hence, a woman who had amniocentesis at 16 weeks can only have termination of pregnancy done at 18-19 weeks, which is later than the 17 weeks allowed in Islam. This poses problems about the type of advice that can be offered to such Muslim parents where detection is too late to allow abortion before the soul breathes.

In addition, some health care practitioners may be opposed to abortion on religious, moral or ethical grounds and hence face difficulties in counselling their patients about screening. In such situations they have an ethical obligation to refer their patients on to a colleague. This may delay screening and detection to allow for early termination of pregnancy.

Thus it can be concluded that there are serious ethical and religious issues in screening of maternal serum.

3.4 Training

Royal College of Obstericians and Gunaecologist stated that there is no statutory requirement for ultrsound practitioner to receive accredited training. It was also suggested that medical staff who undertake ultrasound scanning for foetal abnormality should ideallu hold a advanced Certificate of Ultrasound Training which is issued following a 300 hours course centers recognized by the RCOG/RCR. Skill should be maintained by performing detailed scan one and preferably two sessions a weeks and should not undertake scans of any sort if they have not been specifically trained. In Aberta, college of physician and surgeon statedt that they must have completed a minimum of 6 months full time training in ultrosonography in a teaching program accredited by the Royal College of Physician and Surgeon of Canada. The policy that had been approved by the Diagnostic Imaging Commitee of Society of Obstetricians and Gynaecologist of Canada Training should be carried out in a large accredited service which provides all aspects of obstetrical and gynaecological care and interaction should be with trained supervisors on a one to one basis, the expertise must be achieved in 6 months dedicated training period. This mean that trainee have to participte in 100 gynaecologic and early pregnancy scans and to have participated in at least 200 obstetrical scans covering the full spectrum of obstetrical conditions and must perform 170 ultrasound procedures annually at each obstetrical or focused obstetrical and/or gynaecological sites.

American Registry of Diagnostic Medical Sonographers recommendation for training and continuous medical education that there are no specific recommendation for individual performing ultrasound , however, the physician responsible for the cre of the patients should hve completed ultrasound training in residency or completed a postgraduate course in ultrasound for limited ultrasound evaluation. While for complete ultrasound evaluation it must have 100 supervised scans s part of physician training (DianaEB & Petersen MD 1999)

According guidelines on the use of Ultrasound in Pregnancy Perinatal Society of Malaysia recommendation that the level of expertise required in ultrasound scanning can be divided into 3 levels. There are level 1 (Basic) in which it required to attendance at one basic course and the remainder in an accredited hospital by member of a board run by Obstetric & Gynaecologica Society or Ultrasound in Medicine or Perintal Society of Malaysia or senior members of the obstetric department of the accredited hospital and performing 100 ultrasound scans under supervision. For level 2 (Intermitted) the requirement are attended accredited in intemediate and /or advance course, 1 year experience after basic level accreditation and incorporation into postgraduate training programmes like master in Obstetrics and Gynaecology and psot MRCOG in accredited hospitals. While level 3 (Advanced) must had attended accredited coursed eg RCOG/RCR in foetal Medicine for 1 year or accredited local course, and scholarship in recognized Foetal Medicine training centers abrod such as DDU (Austrlia), RCOG/RCR course accreditation or Mmed (Foetl Medicine) Birmingham.

4. CONCLUSION

There is sufficient evidence to support the use of triple serum marker combined with ultrasound in second trimester, but there are major ethical and religious issues among the Muslim population

5. RECOMMENDATIONS

Due to the ethical and religious issues surrounding prenatal screening, invasive diagnostic procedures and termination of pregnancies, routine antenatal maternal serum screening for Down’s syndrome it is not recommended. However, screening should be made available to women who request for the test.

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EVIDENCE TABLE: DOWN'SS SYNDROME – EFFECTIVENESS

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
BENEFIT OF SCREENING				
1	Wald NJ et al Antenatal maternal serum screening for Down's syndrome: results of a demonstration project. BMJ 1992; 305: 391-4	Prospective trial using a statistical model for predicting Down's syndrome risk on the basis of the three analyses and maternal age and a risk cut-off of 1:250 Sample size 12,603	Uptake of screening 74% Detection rate was 48% False positive rate was 4.1% Uptake of amniocentesis in screen positive cases was 75% Estimated cost of avoiding the birth of a baby with Down's syndrome was 38000 pounds, substantially less than life time cost of care	Poor
2	Haddow JE et al Reducing the need for amniocentesis in women 35 years of age or older with serum markers for screening N Engl J Med 1994; 330:1114-8	Prospective study Sample size 5385 singleton pregnancies who were >=35 years and were undergoing amniocentesis. MSAFP, unconjugated estriol & HCG	If amniocentesis had been reserved for women with risk >1:200 of having a fetus with DS, 48/54(89%) of DS cases would have been detected at 25% false positive rate. 7/15(47%) fetuses with other trisomies , 11/25(44%) with sex aneuploidy, and 1/9(11%) with misc chromosomal abnormalities would have been detected.	Study also compared number of fetuses lost & cost of detecting a DS fetus using the two different protocols
3	Wenstrom KD et al Comparison of multiple-marker screening with amniocentesis for the detection of fetal aneuploidy in women greater or equal to 35 years old. Am J Obstet Gynecol 1995; 173:1287-92	Sample size 1942 women >= 35 years old Prospective data from 1423 women Retrospective data from 519 women Markers: MSAFP, HCG & unconjugated estriol and maternal age) Risk cutoff 1:190	DS screen positive rate was 26.1% (507/1942) DS detection rate was 75% (33/44) Trisomy 18 detection rate was 75% (3/4) Detection of all aneuploidies was only 61%	The multiple-marker screening test fails to detect 39% of all aneuploidies in women >=35.

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
SERUM MARKERS				
1	Merkatz IR et. al An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities Am J Obstet Gynecol, 1984; 148: 886-94	Retrospective study with normal matched controls Sample size 3862 genetic amniocentesis	32 fetal autosomal trisomy detected 9 cases added from another lab Maternal serum alpha-fetoprotein was significantly lower for these 41 women with Trisomies 21, 18 & 13 12 cases of sex chromosomal abnormalities were also detected, 7/12 with serum alpha - fetoprotein level below median.	4-Fair Maternal serum alpha-fetoprotein valuable in improving prenatal detection of autosomal trisomy
2	Borgart MH et. al Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosomal abnormalities Prenat Diagn 1987; 7:623-30	25 abnormal, 74 normal pregnancies between 18-25 weeks pregnancy	Determination of elevated levels of hCG or alpha hCG detected 68% of pregnancies with chromosomally abnormal fetuses with a false positive rate of 1.35% Determination of both elevated and depressed levels of HCG detected 76% of chromosomally abnormal fetuses with 4.05% false positive rate	4-Fair Total hCG or alpha hCG useful in detecting pregnancies with chromosomally abnormal fetuses
3	Wald NJ et.al. Maternal serum unconjugated oestriol as an antenatal screening test for Down's Syndrome Br J Obstet Gynaecol 1988; 95:334-341	Retrospective study of stored samples in 77 pregnancies associated with Down's Syndrome and 385 unaffected controls	The median level for affected pregnancies was 73% of that in control	4-Fair uE_3 is useful to detect Down's syndrome pregnancies
4	Cuckle H et. al Antenatal screening for Down's syndrome BMJ 1992;305:1017 (Letter to the editor)	9 references including meta-analysis	Free beta-hCG gives an 11-12% higher detection rate compared to total hCG, with only a 0.4-0.7% increase in false positive rate. Unlike total HCG, it can be used for screening before 15 weeks and screening can be offered from 13 weeks.	Free beta subunit of HCG is preferred to total HCG Screening can be offered from 13 weeks.

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
5	Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. Health Technology Assessment 1998; 2(1)	Review of the performance of the 6 serum markers between 15-22 weeks in a total of 63 studies **Serum AFP** 38 studies, n=1328 ***uE₃*** 21 studies, n=733 ***hCG*** 28 studies, n=907 **Free alpha-hCG** 7 studies, n=239 **Free beta-hCG** 12 studies, n=562 **Dimeric inhibin A** 6 studies, n=375	Pooled median serum AFP (MoM) at 15-22 weeks was 0.75. 95% CI (0.72-0.78) Pooled median uE_3 (MoM)at 15-22 weeks was 0.72. 95% CI (0.68-0.75) Pooled median hCG (MoM) at 15-22 weeks was 2.06. 95% CI (1.95-2.17) Pooled median free alpha-hCG (MoM) at 15-22 weeks was 1.43 95% CI (1.12-1.82) Pooled median free beta-hCG (MoM) at 15-22 weeks was 2.20 95% CI (2.07-2.33) Pooled median dimeric inhibin A (MoM) at 15-22 weeks was 1.92 95% CI (1.75-2.15)	Serum AFP useful. uE_3 useful Total hCG useful Free alpha- hCG useful Free beta- hCG useful Dimeric inhibin A useful
6	Wald NJ, Kennard A, Hackshaw A, McGuire A. Antenatal screening for Down's syndrome. Health Technol Assessment 1998; 2(1)	Review of the performance of other serum markers between 15-22 weeks in a total of 21 studies **CA 125** 5 studies, n=81 **PAPP-A** 3 studies, n=64 **SP 1** 7 studies, n=379 **Alpha-inhibin**	Pooled median CA 125 (MoM) at 15-22 weeks was 0.94 95% CI (0.74-1.21) Pooled median PAPP-A (MoM) at 15-22 weeks was 0.97 95% CI (0.84-1.11) Pooled median SP 1 (MoM) at 15-22 weeks was 1.47 95% CI (1.23-1.76) Pooled median alpha-inhibin (MoM) at 15-22 weeks was 1.63 95% CI (1.01-2.62)	CA 125 not useful PAPP-A not useful SP 1 has limited value Alpha-inhibin has limited value

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
		4 studies, n=64		
7	Wald NJ et al Maternal serum screening for Down's syndrome in early pregnancy BMJ 1988; 297:883-7	Retrospective study of storedsamples in 77 pregnancies associated with Down's Syndrome and 385 unaffected controls	A screening programme combining maternal age, serum AFP, unconjugated oestriol and HCG could detect over 60% of affected pregnancies with 5% amniocentesis rate. It is the most cost effective way to screen.	It is illogical to offer amniocentesis to all women above a certain age and to restrict the use of biochemical tests to younger women. If such a selective approach were adopted some older women with a fairly low risk would have an amniocentesis while some younger women with a higher risk would not.
8	Macri JN et al. Maternal serum free beta hCG screening: results of studies including 480 cases of Down's syndrome. Prenatal Diagnosis 1994;14(2):97-103	In 234 of these cases from retrospective and prospective studies, the effectiveness of maternal serum free beta hCG was evaluated in combination with alpha-fetoprotein (AFP) and maternal age in second-trimester Down's syndrome screening.	Down's syndrome detection in the gestational age range of 14-16 weeks was 82 per cent. In all gestational weeks (14-22), a 77.7 per cent Down's syndrome detection rate was achieved. In prospective screening of 44,272 patients under the age of 35 years, 69 per cent of Down's syndrome cases were detected (73 per cent in gestational weeks 14-16). The false-positive rate for the prospective study was 3.8 per cent. The use of free beta hCG combined with maternal serum AFP and maternal age-related risk for Down's syndrome in a screening population (i.e., women under 35 years) yields an improved detection efficiency over other protocols.	
9	Spencer K. Free alpha-subunit of Human	Case control studies 36 cases of DS pregnancies compared with 180 normal	Free alpha –subunit of HCG concentrations in the DS group were not significantly different from those in the unaffected group.	7-Poor

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	Chorionic Gonadotropin in Down's Syndrome Am J Obstet Gynecol 1993; 168(1):132-135	controls within gestation 16-20 weeks. 100% follow-up	A case for using this subunit of HCG in DS screening protocols is not yet proved.	
10	Saller DN et al. Multiple marker screening in pregnancies with hydropic and nonhydropic Turner syndrome Am J Obstet Gynecol 1992; 167:1021-4	Descriptive study Pre-amniocentesis second trimester maternal serum specimens from 7 hydropic & 8 non-hydropic fetal Turner syndrome pregnancies	In both hydropic & non-hydropic cases, AFP were slightly reduced. UE3 were markedly reduced.. Hydropic pregnancies had elevated HCG levels. Non-hydropic pregnancies had low HCG levels	
11	Wenstrom KD et al Detection of fetal Turner Syndrome with multiple marker screening Am J Obstet Gynecol 1994; 170:570-73	Retrospective review Sample size 27,282 screening tests (triple tests and maternal age) Risk cut-off for Down's Syndrome >=1:190	5 of the 17 expected cases of Turner syndrome were identified with the screening test alone (29%)	
FIRST TRIMESTER SCREENING				
1	Crandall BF et al Maternal serum screening for alpha-fetoprotein, unconjugated estriol, and human chorionic gonadotropin between 11 and 15 weeks of pregnancy to detect fetal chromosomal abnormalities Am J Obstet Gynecol 1993; 168:1864-9	Non-controlled clinical series Sample size 993 (90% >=35, 10%<35) Cut-off risk for DS 1:365	9 of 11 (82%) DS pregnancies identified. 23% false positive results in women >=35, 6% in women <35 12 other unbalanced chromosomal abnormalities, 2 in women <35, 10 in women >=35. Only three cases had abnormal triple tests. 12 had inherited balanced chromosomal rearrangements or variants; all had normal screening tests.	8-poor Sample size too small to draw universal conclusions about maternal serum screening between 11-15 weeks. Most other chromosomal abnormalities will be missed. Women seeking prenatal

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
				testing because of advanced maternal age should be advised of the limitations of the tests.
2	Krantz DA et al First-trimester Down's syndrome screening: Free beta-HCG and PAPP-A Am J Obstet Gynecol 1996; 174:612-6	Retrospective study 22 DS cases & 483 controls	At a 5% false-positive rate, 63% of cases could be expected to be detected First trimester free beta-HCG & PAPP-A screening for DS can achieve detection rates as high as those associated with triple markers in the second trimester.	4-Fair Unable to detect NTD Findings need to e confirmed by prospective studies.
3	Wald NJ, Kennard A, Hackshaw A, McGuire A Antenatal screening for Down's syndrome. Health Technol Assessment 1998; 2(1)	Review of the performance of serum markers between 10-14 weeks **Serum PAPP-A** 12 studies, n=297 **Free beta-hCG** 12 studies, n=308	Pooled median serum PAPP-A (MoM) at 10-14 weeks was 0.38 95% CI (0.33-0.43) Pooled median free beta-hCG (MoM) at 10-14 weeks was 1.83 95% CI (1.65-2.03) Maternal age combined with PAPP-A and free beta-hCG gives a 62% detection rate for a 5% false-positive rate. Combined with nuchal translucency measurement improves the detection rate to 80% for 5% FPR	1-Good Serum PAPP-A and free beta-hCG are useful markers. Screening performance comparable with second trimester screening using triple markers. However 1:4 affected pregnancies will spontaneously abort between 1st &2nd trimester.
4	Malone FD et al First trimester screening for aneuploidy: Research or standard of care?	Review article 40 references	Nuchal translucency on ultrasonography forms the basis of this new form of screening but studies of its efficacy have yielded widely conflicting results, with detection rates ranging from 29%-91%	1-Good The current standard of care with respect to DS screening should not be changed, and first

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	Am J Obstet Gynecol 2000; 182:490-96		Studies using PAPP –A & free beta HCG have been more consistent yielding 55%-63% detection rates at 5% FPR. Combination of first trimester US & serum screening has not been adequately studied.	trimester screening should remain investigational.
5	Stranc LC Chorionic villus sampling and amniocentesis for prenatal diagnosis Lancet 1997; 349”711-17	Correspondance	Cordocentesis is usually done after 18 weeks of gestation in women presenting late. In addition, it has been said that amniocentesis in the first trimester is still considered an experimental technique if carried out at 11-14 weeks.	
SCREENING AT 15-22 WEEKS OF PREGNANCY				
1	Wald NJ et al Antenatal maternal serum screening for Down’s syndrome: results of a demonstration project. BMJ 1992; 305: 391-4	Prospective trial using a statistical model for predicting Down’s syndrome risk on the basis of the three analyses and maternal age and a risk cut-off of 1:250 Sample size 12,603	Uptake of screening 74% Detection rate was 48% False positive rate was 4.1% Uptake of amniocentesis in screen positive cases was 75% Estimated cost of avoiding the birth of a baby with Down’s syndrome was 38000 pounds, substantially less than life time cost of care	
2	Haddow JE et al. Prenatal screening for Down’s syndrome with use of maternal serum markers N Eng J Med 1992; 327:588-93	Prospective trial Sample size 25,207 Risk cut-off 1:190	6.6% of women screened were identified as having Down’s syndrome risk of >=1:190 The rate of screen positive women were reduced to 3.8% when ultrasound was used to establish gestation 21 of the expected 36 cases were detected (58%). 7 cases of other chromosomal disorders were detected	
3	Phillips OP et al. Maternal serum screening for fetal Down’s syndrome in women less than 35 years of age using alpha-	Prospective study Sample size 9530 between 15-20 weeks gestation	Initial positive screening rate of 7.2% (686) Ultrasonographic exam explained the abnormal values in 379(4.0%)	Abstract only

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	fetoprotein, hCG, and unconjugated estriol: a prospective 2-year study. Obstet Gynecol 1992; 80:353-8	Risk cut-off 1:274	The remaining 307 (3.2%) had genetic counseling and 214 (2.2%) opted for invasive prenatal tests 4 of 7 cases of Down's syndrome were identified (57%)	
5	Goodburn SF et al Second trimester maternal serum screening using alpha-fetoprotein, human chorionic gonadotrophin, and unconjugated oestriol: experience of a regional programme Prenat Diagn 1994; 14:391-402	Prospective study 25,359 singleton pregnancies	75% detection rate for Trisomy 21 with triple marker screening compared with 52% when unconjugated oestriol was excluded. Other fetal abnormalities detected were trisomy 18 (3), trisomy 13 (2), 45,X (6), 69,XXX (5), other chromosome abnormalities (9), open neural tube defects (26), hydrocephalus (7), abdominal wall defects (4), and steroid sulphatase deficiency (6).	
6	Benn PA et al Fetus-Placenta-Newborn:Prenatal diagnosis of diverse chromosomal abnormalities in a population of patients identified by triple-marker testing as screen positive for Down's syndrome Am J Obstet Gynecol 1995; 173:496-501	Prospective trial Sample size 11, 434 patients between 15-22 weeks gestation Risk cutoff 1:270	Screen positive rate 5.92%. Karyotypes reviewed in 69% 14/20 (70%) of DS were identified. 12 other chromosomal abnormalities were found, 5 of whom had sex chromosomal abnormalities. 2 were 45,X 23 cases of sex chromosomal abnormalities should be present. Triple marker testing does not, in general, result in a strong preferential identification of sex chromosome abnormalities (other than 45,X).	Approximately 5% of prenatal diagnoses on those patients screen positive for DS will show some type of chromosome abnormality. Although serum screening reports emphasize detection of DS and Trisomy 18, preamniocentesis counseling should reflect the fact that a variety of chromosomal abnormalities will be identified.
7	Burton BK et al .A prospective trial of prenatal	Prospective trial Sample size 8233 midtrimester	10.4% were found to have positive screen results for DS	4-fair Prenatal screening

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	screening for Down's syndrome by means of maternal serum alpha-fetoprotein, human chorionic gonadotropin, and unconjugated estriol Am J Obstet Gynecol 1993; 169:526-30	serum samples 7492 from women <35 years old 741 >=35 years old Risk cut-off 1:270	329 had amniocentesis because of DS risk >=1:270. 12 (1 in 33) had a fetus with chromosomal anomaly. 8 were Down's syndrome, 3 Turner syndrome,1 with a balanced 13/14 translocation. 2 DS cases were born to screen positive mothers who had refused amniocentesis. 2 DS cases were missed by screening. 10 of 12 (83%) would have been detected. Of 0.4% of patients at increased risk for Trisomy 18 (all three markers were low) , 2 cases of Trisomy 18 & 1 triploidy were found. 3 of the 5 cases of 45,X were detected in patients with a DS risk of >=1:270	with AFP, hCG, and unconjugated estriol is an effective method for identification of pregnancies at high risk for Down's syndrome. A significant percentage of 45,X cases will be detected through triple marker screening
8	Kellner LH et al. Fetus-Placenta-Newborn:The advantages of using triple-marker screening for chromosomal abnormalities Am J Obstet Gynecol 1995; 172:831-836	Prospective study 10,605 samples between 15-22 weeks' gestation A second trimester risk for trisomy >= 1:270 was considered positive Screen positive for trisomy 18 if all three markers were low:AFP<=0.75MoM, uE3<=0.60MoM, HCG<=0.55MoM.	Initial screen positive rate was 8.3% 12 of 16 ascertained cases of trisomy 21(75%), 2 of 3 cases of trisomy 18(67%), 5 cases of 45,X karyotype, one each of 45,X/46,XX, 47XXY, 47XYY, 46,XX,ins(2)(q21p13p15)mat, and 69,XXX karyotypes were identified in the screen-positive patients. Omitting uE3 would have resulted in detection of 9 of the 16 trisomy 21 and 6 of the other chromosomal abnormalities with the same false positive rate.	
9	Kellner LH et al. Triple marker (alpha-fetoprotein, unconjugated estriol, human chorionic gonadotropin) versus alpha-fetoprotein plus free-beta	Free beta HCG was concurrently assayed in 2349 maternal serum samples. Free beta HCG from 12 cases of fetal DS previously	Screen positive rate with the triple marker was 8.0% compared with 12.8% for alpha-fetoprotein plus free beta HCG 12/15 (80%) were detected with triple marker	The detection of fetal DS was greater by use of a triple marker screen than when using alpha fetoprotein plus free beta HCG.

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	subunit in second trimester maternal serum screening for fetal Down's syndrome: A prospective comparison study Am J Obstet Gynecol 1995; 173:1306-1309	identified with triple markers was retrospectively assayed	9/15 (60%) were detected with the two markers	
10	Rose NC et al Maternal serum alpha-fetoprotein screening for chromosomal abnormalities: a prospective study in women aged 35 and older Am J Obstet Gynecol 1994; 170:1073-80	Prospective study 3896 women had serum maternal alpha-fetoprotein measured routinely before amniocentesis for the indication of advanced maternal age. Risk cut-off 1:270	63% of unaffected pregnancies had positive screening tests 85% of fetal Down's syndrome pregnancies had second trimester risks of >=1:270 51.9% of other chromosomal abnormalities were also assigned risks of >=1:270 Maternal serum alpha-fetoprotein screening is more accurate than age alone for assigning individual DS risk in pregnant women >=35 years old.	4-Fair Using triple markers in this group will be able to further reduce the false positive rate.
7	Wald NJ et al Maternal serum screening for Down's syndrome in early pregnancy BMJ 1988; 297:883-7	Retrospective study of storedsamples in 77 pregnancies associated with Down's Syndrome and 385 unaffected controls	The median level for affected pregnancies was 73% of that in control	4 Fair uE_3 is useful to detect Down's syndrome pregnancies
SCREENING IN WOMEN AGED 35 YEARS OR ABOVE				
1	Haddow JE et al Reducing the need for amniocentesis in women 35 years of age or older with serum markers	Prospective study Sample size 5385 singleton pregnancies who were >=35 years and were undergoing	If amniocentesis had been reserved for women with risk >1:200 of having a fetus with DS, 48/54(89%) of DS cases would have been detected at 25% false positive rate.	Study also compared number of fetuses lost & cost of detecting a DS fetus using the two different protocols

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	for screening N Engl J Med 1994; 330:1114-8	amniocentesis. MSAFP, unconjugated estriol & HCG	7/15(47%) fetuses with other trisomies , 11/25(44%) with sex aneuploidy, and 1/9(11%) with misc chromosomal abnormalities would have been detected.	
2	Wenstrom KD et al Comparison of multiple-marker screening with amniocentesis for the detection of fetal aneuploidy in women greater or equal to 35 years old. Am J Obstet Gynecol 1995; 173:1287-92	Sample size 1942 women >= 35 years old Prospective data from 1423 women Retrospective data from 519 women Markers: MSAFP, HCG & unconjugated estriol and maternal age) Risk cutoff 1:190	DS screen positive rate was 26.1% (507/1942) DS detection rate was 75% (33/44) Trisomy 18 detection rate was 75% (3/4) Detection of all aneuploidies was only 61%	The multiple-marker screening test fails to detect 39% of all aneuploidies in women >=35.
3	Rose NC et al Maternal serum alpha-fetoprotein screening for chromosomal abnormalities: a prospective study in women aged 35 and older Am J Obstet Gynecol 1994; 170:1073-80	Prospective study 3896 women had serum maternal alpha-fetoprotein measured routinely before amniocentesis for the indication of advanced maternal age. Risk cut-off 1:270	63% of unaffected pregnancies had positive screening tests 85% of fetal Down's syndrome pregnancies had second trimester risks of >=1:270 51.9% of other chromosomal abnormalities were also assigned risks of >=1:270 Maternal serum alpha-fetoprotein screening is more accurate than age alone for assigning individual DS risk in pregnant women >=35 years old.	4-Fair Using triple markers in this group will be able to further reduce the false positive rate.
AMINOCENTESIS AND CHORIONIC VILLI SAMPLING				
1	Smidt-Jensen et al. Randomised comparison of amniocentesis and transabdominal and transcervical chorionic villus sampling.	RCT 3079 women at low genetic risk CVS approaches were	Among 3079 women at low genetic risk total fetal loss rates were 10.9% for TC CVS, 6.3% for TA CVS, and 6.4% for AC ($p < 0.001$). More women had bleeding after the procedure in the CVS groups ($p < 0.001$), whereas more amniotic fluid	2-Good The risk of fetal loss is similar after TA CVS and AC. TC CVS associated

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	Lancet 1992; 340(8830):1237-44	compared among 2882 women at low and high genetic risk who were found to have cytogenetically normal fetuses	leakage ($p < 0.001$) was reported after AC. No uterine infections occurred in any group. No case of oromandibular-limb abnormality was seen in the CVS groups, but 1 child in the AC group had aplasia of the right hand. The two CVS approaches were compared among 2882 women at low and high genetic risk who were found to have cytogenetically normal fetuses. Rates of unintentional loss after the procedure were 7.7% for TC CVS and 3.7% for TA CVS ($p < 0.001$; 95% Cl of difference 2.3-5.8%).	with higher fetal loss rate compared with TA CVS
2	Smidt-Jensen S et al. Comparison of transabdominal and transcervical CVS and amniocentesis: sampling success and risk. Prenatal Diagnosis 1991; 11(8):529-37 (abstract)	RCT Sample size 2931	No significant difference was seen between total fetal loss in the transabdominal CVS group and the amniocentesis group (6.5 and 6.8 per cent, respectively, SE difference = 0.92 per cent, $p = 0.01$). The total fetal loss in the transcervical CVS group was 10.1 per cent.	2-Good No significant difference was seen between total fetal loss in the transabdominal CVS group and the amniocentesis group. Transcervical CVS associated with higher fetal loss rate.
THE ROLE OF ULTRASONOGRAPHIC EXAMINATION				
1	Wald NJ et al Maternal serum screening for Down's syndrome: the effect of routine ultrasound scan determination of gestational age and adjustment for maternal weight. .Br J Obstet Gynaecol 1992;	Prospective study 2113 women with a singleton pregnancy without Down's syndrome	The routine use of ultrasound to estimate gestational age will increase the detection rate from 58% to 67% while maintaining the false positive rate at 5%, or reduce the false positive rate from 5.7% to 3.1% while maintaining the detection rate at 60%	4-Fair Dating gestation using ultrasound will improve screening performance serum markers.

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	99:144-9			
2	Benn PA et al. Down's syndrome and neural tube defect screening: The value of using gestational age by ultrasonography Am J Obstet Gynecol 1997; 176:1056-1061	Retrospective study Study population >24,000 women at 15.0-21.9 weeks' gestation. Initial & revised screen-positive rates and detection rates were reviewed for women undergoing triple marker testing. Gestational age & screening results were compared for 24 Down's syndrome pregnancies	Both initial & revised screen-positive rates for DS were significantly lower when U/S data were used compared with LMP. Detection rate for DS appeared to be higher with U/S dating (76% vs 60%)	4-Fair When ultrasonographic information is available it should be preferentially used over LMP data because it significantly improves the effectiveness of serum screening
3	Benacerraf BR et al Sonographic diagnosis of Down's syndrome in the second trimester Am J Obstet Gynecol 1985; 153:49-52	800 cases in prospective series 904 cases in retrospective series	11 cases of Down's syndrome were diagnosed cytogenetically 5/11 (45%) had increased skin/soft tissue thickening at the back of the fetal neck suggestive of DS	3-Fair Increased nuchal skin fold thickness is a valuable marker. Anomaly scan has potential value in the screening programmed
4	Abuhamad AZ et al Ultrasonographic fetal iliac length measurement in the screening for Down's syndrome Am J Obstet Gynecol. 1994; 171:1063-67	Prospective study 10 fetuses with Down's syndrome and 180 karyotypically normal fetuses before genetic amniocentesis.	Iliac length measurement in Down's syndrome fetuses was significantly longer than in normal controls. A ratio of 1.21 for observed-to-expected iliac length measurement yielded a sensitivity of 40%, a specificity of 98%, positive predictive value of 1/38 in a low risk population with a false-positive rate of 2%.	3-Fair Iliac length measurement may be useful as an ancillary screening variable in antenatal screening for Down's syndrome.
5	Johnson MP et al Fetal leg and femur/foot length ratio: A marker for Trisomy 21	Retrospective study of direct necropsy measurement in 391 normal pregnancies & 45 with trisomy 21.	A leg/foot length ratio versus gestation age <= 2.3 correctly identified 84% of fetuses with trisomy 21 between 110 to 155 days gestation age at necropsy. An ultrasonographic femur/foot length ratio versus	3-Fair The femur/foot length ratio is an additional ultrasonographic marker for

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	Am J Obstet Gynecol 1993; 169:557-63	Prospective study involving ultrasonographic measurement for femur/foot length ratio versus gestational age in 345 midgestational pregnancies.	gestation age <= 0.9 in the high-risk population correctly identified 71% of fetuses with trisomy 21.	identification of fetuses at increased risk for trisomy 21.
6	Johnson MP et al Fetus-Placenta-Newborn: Combining humerus and femur length for improved ultrasonographic identification of pregnancies at increased risk for trisomy 21. Am J Obstet Gynecol 1995; 172:1229-35	Retrospective study of direct necropsy measurement in 641 normal pregnancies & 62 with trisomy 21. Prospective study involving ultrasonographic measurement for (femur+humerus length)/foot length ratio in 576 midgestational pregnancies.	An ultrasonographic (femur + humerus length)/foot length ratio <=1.75 gave a 15.3 odds risk for trisomy 21 in the high risk population and correctly identified 53% of fetuses with trisomy 21 with a FPR of 7%.	3-Fair The (femur + humerus length)/foot length ratio may be an aadditional effective ultrasonographic marker for identification of fetuses at increased risk forvtrisomy 21.
7	Yagel S et al. The role of midtrimester targeted fetal organ screening combined with the “triple test” and maternal age in the diagnosis of trisomy 21: A retrospective study Am J Obstet Gynecol 1998; 178:40-44	Retrospective study of all Trisomy 21 cases diagnosed prenatally & postnatally between Jan 1990 and Dec 1993 in Jerusalem	Ultrasonographic examination at midtrimester increased prenatal diagnosis of trisomy 21 by 30% in women < 35 years old and by 10.7% in the total population.	4-Fair The role of ultrasonographic examination as a primary screening tool for the prenatal diagnosis of DS cannot be evaluated from this study. However in mothers < 35 years with normal triple test, ultrasonographic examination at midtrimester increased prenatal diagnosis of trisomy 21 by 30%
8	Owen J. et al	Retrospective study.	The relative difference in the femur length ratio between normal and affected fetuses was small in	3-Fair The addition of

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	The utility of fetal biometry as an adjunct to the multiple marker screening test for Down's syndrome. Am J Obstet Gynecol. 1994; 171:1041-46	38 cases of Down's syndrome and 1098 euploid controls.	comparison to that of the maternal serum analytes.	measured/predicted femur length ratio had a negligible effect on the performance of the multiple-marker screening test.
9	Bahado S et al Risk of Down's syndrome and any clinically significant chromosome defect in pregnancies with abnormal triple screen and normal targeted ultrasonographic results. Am J Obstet Gynecol. 1996; 175:824-29	Prospective study of 1034 cases at risk of Down's syndrome or trisomy 18 based on triple marker screening. Ultrasonographic examination performed between 15-24 weeks gestation.	11 cases of DS, 1 of trisomy 18, 17 clinically significant chromosome defects. Abnormal nuchal thickness or observed/expected combined femur and humerus length < 0.92 was the most parameter for Down's syndrome detection. In pregnancy with a 1:270 triple-screen risk for DS, normal biometric and anatomic results reduce the risk to 1 in 2100.	3-Fair Normal ultrasonographic anatomy and biometry significantly reduces the risk of Down's syndrome.
10	Ginsberg N et al. Abnormal triple-test result plus normal ultrasonographic results equal amniocentesis? (Letters to the Editors) Am J Obstet Gynecol		Even in the best of hands, only 50% of the fetuses with DS have ultrasonographic abnormalities.	Ultrasound should not be used as a secondary test in women with positive serum screening results.
11	Nyberg DA et al Role of prenatal ultrasonography in women with positive screen for Down's syndrome on the basis of maternal serum markers. Am J Obstet Gynecol. 1995; 173:1030-1035	Prospective study 395 women who were screened positive by triple marker screening. Ultrasonographic abnormalities that were evaluated included structural defects, nuchal thickening or cystic hygroma, echogenic	Among 395 patients, 374(94.7%) had normal karyotype, 18 (4.5%) had Down's syndrome, 3 had other karyotypic abnormalities. One or more ultrasonographic abnormalities were found in 9 of 18 (50%) with Down's syndrome compared to 7.2% in other fetuses. An abnormal ultrasonography result increased the risk of Down's syndrome by 5.6-fold (25% from 4.5%) and	3-Fai Abnormal ultrasonographic findings increase the risk of Down's syndrome, whereas normal findings are less predictive of normalcy. After correction for

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
		bowel, cerebral ventricular dilatation, pyelectasis and shortened femur.	a negative result reduced the risk by 45% (2.5% from 4.5%).	inaccurate menstrual dates, genetic amniocentesis should be offered in spite of a normal ultrasonography result among women with positive triple screen.
12	Wald NJ et al Antenatal screening for Down's syndrome. Health Technol Assessment 1998; 2(1)	Literature review	Nuchal fold thickness 16 studies, DR 38%, FPR 1.3% *Femur length (comparing observed with expected)* 10 studies, DR 34%, FPR 5.9% Femur length (ratio of BPD to femur length) 4 studies, DR 22%, FPR 5.9% Humerus length (comparing observed with expected) 6 studies, DR 37%, FPR 5.3% Femur length and humerus length combined (comparing observed with expected) 3 studies, DR 36%, FPR 3.7% Pyelectasis 4 studies, DR 19%, FPR 2.4% Hyperechogenic bowels 3 studies, DR 11%, FPR 0.7%	1-Good Nuchal fold thickness has the best detection rate.
13	Smith-Bindman R et al Second trimester ultrasound to detect fetuses with Down's syndrome: A meta-analysis (review)	A meta-analysis of 56 articles (between 1980 & Feb 1999) describing 1930 fetuses with Down's syndrome and 130 365 unaffected fetuses Markers studied: choroids	When U/S markers were observed without fetal structural malformations, sensitivity for each was (range, 1-16%), and most fetuses with such markers had normal outcomes. A thickened nuchal fold was the most accurate marker for discriminating between unaffected and affected	1-Good A thickened nuchal fold in the second trimester may be useful in distinguishing unaffected fetuses from those with DS, but the

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & Commment
	JAMA 2001;285:1044-055	plexus cyst, thickened nuchal fold, echogenic intracardiac focus, echogenic bowel, renal pyelectasis, and humeral and femoral shortening.	fetuses and was associated with an approximately 17-fold increased risk of DS. For each of the other 6 markers, when observed without associated structural malformations, the marker had marginal impact on the risk of DS. Because the markers were detected in only a small number of affected fetuses, the likelihood of DS did not decrease substantially after normal examination findings.	overall sensitivity of this finding is too low for it to be a practical screening test for DS. Using these markers as a basis for deciding to offer amniocentesis will result in more fetal losses than cases of DS detected, and will lead to a decrease in the prenatal detection of fetuses with DS.

EVIDENCE TABLE-SAFETY

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & comment
SAFETY OF AMNIOCENTESIS				
1	Tabor A et al. Randomised controlled trial of genetic amniocentesis in 4606 low-risk women. Lancet 1986; 1(8493): 1287-93	RCT Sample size 4606	Spontaneous abortion rate was 1.7% in the study group after amniocentesis and 0.7% in the control group after ultrasound (relative risk 2.3). In the first six weeks after amniocentesis/ultrasound scan, amniotic fluid leakage occurred more often in the study group but there was no difference in the rate of vaginal bleeding. Frequency of postural malformations in the infants in the two groups was the same. In the study group, respiratory distress syndrome was diagnosed more often (relative risk 2.1) and more babies were treated for pneumonia (relative risk 2.5).	2-Good Procedure related fetal loss rate for amniocentesis is 1.0% Increased incidence of respiratory distress syndrome and congenital pneumonia
2	NICHHD National Registry for Amniocentesis Study Group.	Prospective study	Immediate complications of amniocentesis (vaginal bleeding or amniotic fluid leakage) occurred in	3-Fair Midtrimester

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & comment
	Midtrimester amniocentesis for prenatal diagnosis. Safety and accuracy. JAMA 1976; 236(13):1471-6	Sample size 1,040 992 controls	approximately 2% of the women. There was no statistically significant difference between the two groups in rate of fetal loss (3.5% for the subjects, 3.2% for the controls). Newborn examination indicated no significant differences between the two groups in incidence of congenital anomalies and no evidence of physical injury resulting from amniocentesis. The two groups did not differ significantly in physical, neurological, or developmental status at one year of age.	amniocentesis is safe procedure that does not significantly increase the risk of fetal loss or injury.
SAFETY OF CHORIONIC VILLI SAMPLING				
1	Smidt-Jensen et al. Randomised comparison of amniocentesis and transabdominal and transcervical chorionic villus sampling. Lancet 1992; 340(8830):1237-44	RCT 3079 women at low genetic risk CVS approaches were compared among 2882 women at low and high genetic risk who were found to have cytogenetically normal fetuses	Among 3079 women at low genetic risk total fetal loss rates were 10.9% for TC CVS, 6.3% for TA CVS, and 6.4% for AC ($p < 0.001$). More women had bleeding after the procedure in the CVS groups ($p < 0.001$), whereas more amniotic fluid leakage ($p < 0.001$) was reported after AC. No uterine infections occurred in any group. No case of oromandibular-limb abnormality was seen in the CVS groups, but 1 child in the AC group had aplasia of the right hand. The two CVS approaches were compared among 2882 women at low and high genetic risk who were found to have cytogenetically normal fetuses. Rates of unintentional loss after the procedure were 7.7% for TC CVS and 3.7% for TA CVS ($p < 0.001$; 95% Cl of difference 2.3-5.8%).	2-Good The risk of fetal loss is similar after TA CVS and AC. TC CVS associated with higher fetal loss rate compared with TA CVS
2	Smidt-Jensen S et al.	RCT	No significant difference was seen between total fetal	2-Good

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & comment
	Comparison of transabdominal and transcervical CVS and amniocentesis: sampling success and risk. Prenatal Diagnosis 1991; 11(8):529-37 (abstract)	Sample size 2931	loss in the transabdominal CVS group and the amniocentesis group (6.5 and 6.8 per cent, respectively, SE difference = 0.92 per cent, p = 0.01). The total fetal loss in the transcervical CVS group was 10.1 per cent.	No significant difference was seen between total fetal loss in the transabdominal CVS group and the amniocentesis group. Transcervical CVS associated with higher fetal loss rate.
3	Rhoads GG et al. The safety and efficacy of chorionic villus sampling for early prenatal diagnosis of cytogenetic abnormalities. New England Journal of Medicine 1989; 320(10):609-17	Multicentre study 2278 underwent chorionic villus sampling 671 underwent amniocentesis at 16 weeks' gestation	The rate of combined losses due to spontaneous and missed abortions, termination of abnormal pregnancies, stillbirths, and neonatal deaths was 7.2 percent in the group that underwent chorionic villus sampling and 5.7 percent in the group that had amniocentesis. After adjustment for slight differences in gestational and maternal age, the total loss rate for the women in the chorionic villus sampling group exceeded that for the amniocentesis group by only 0.8 percentage points (80 percent confidence interval, -0.6 to 2.2). There were no serious maternal infections among the women in this study or among an additional 1990 women who underwent chorionic villus sampling (upper 95 percent confidence limit, 0.08 percent).	Chorionic villus sampling is a safe and effective technique for the early prenatal diagnosis of cytogenetic abnormalities, but that it probably entails a slightly higher risk of procedure failure and of fetal loss than does amniocentesis
4	Firth HV et al Severe limb abnormalities after chorion villus sampling at 56-66 days' gestation. Lancet 1991; 337(8744):762-3	Sample size 289	5 babies with severe limb abnormalities were subsequently identified. 4 had oromandibular-limb hypogenesis syndromes, and the other had a terminal transverse limb reduction defect.	This high incidence raises the possibility that CVS was an aetiological factor for limb abnormalities
5	Burton BK et al Limb anomalies associated with	Sample size 463 women 394 fetuses and infants who	3.3% had major congenital anomalies, including four with transverse limb reduction deformities, three with cleft lip with or without cleft palate, and one each with	There is an increased risk of limb anomalies associated with CVS.

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & comment
	chorionic villus sampling. Obstetrics & Gynecology. 1992;79(5 (Pt 1)):726-30	were adequately evaluated	a nasal encephalocele, large port-wine stain, craniosynostosis, omphalocele with associated defects, ambiguous genitalia, and undescended testes. The procedures were performed at 9.5, 9.5, 10.5, and 11 weeks' gestation in the 4 cases with limb abnormalities.	
6	US Preventive Task Force Report Guide to Clinical Preventive Service 2nd Edition 1996	Technical Report	Current estimates for the overall risk of transverse limb deficiency from CVS range from 0.03% to 10% of procedure.	
SAFETY OF TERMINATION OF PREGANANCY				
1	Hakim-Elahi E et al. Complications of first-trimester abortion: a report of 170,000 cases. Obstetrics & Gynecology 1990; 76:129-35	Sample size 170,000 first trimester abortions	There were no deaths in this series of patients. One hundred twenty-one patients were hospitalized (0.71 per 1000) for suspected perforation, ectopic pregnancy, hemorrhage, sepsis, or recognized incomplete abortion. There was no major extirpative surgery performed. There were an additional 1438 minor complications (8.46 per 1000). Overall, there were 9.05 complications per 1000 abortions.	
2	Lawson HW et al Abortion mortality, United States, 1972 through 1987. AM J of Obstet & Gynecol 1994; 71:1365-72	Retrospective review of abortion mortality surveillance data collected by the Division of Reproductive Health, CDC and Prevention. Rates are reported as legal abortion deaths per 100,000 abortions.	Between 1972 and 1987, 240 women died as a result of legal induced abortions. The case-fatality rate decreased 90% over time, from 4.1 deaths per 100,000 abortions in 1972 to 0.4 in 1987. Women > or = 40 years old had three times the risk of death as teenagers (relative risk 3.0, 95% confidence interval 1.5 to 6.0). Black women and those of other minority races had 2.5 times the risk of white women (relative risk 2.5, 95%	Legal induced abortion-related deaths are rare events. Mortality is higher in second trimester abortion compared to first trimester abortion.

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & comment
			confidence interval 1.9 to 3.2). Abortions at > or = 16 weeks were associated with a risk of death almost 15 times the risk of death from procedures at < or = 12 weeks' gestation. Women undergoing currettage procedures for abortion had a significantly lower risk of death than women undergoing other procedures. Whereas before 1977 infection and hemorrhage were the leading causes of death, during 1977 through 1982 anesthesia complications emerged as one of the leading causes of death and since 1983 have become the most frequent cause.	
3	American Medical Association. Induced termination of pregnancy before and after Roe v Wade. Trends in the mortality and morbidity of women. Council on Scientific Affairs. JAMA 1992; 268:3231-9	The mortality and morbidity of women who terminated their pregnancy before the 1973 Supreme Court decision in Roe v Wade are compared with post-Roe v Wade mortality and morbidity. Mortality data before 1973 are from the National Center for Health Statistics; data from 1973 through 1985 are from the Centers for Disease Control and The Alan Guttmacher Institute.	Deaths from legal abortion declined fivefold between 1973 and 1985 (from 3.3 deaths to 0.4 death per 100,000 procedures), reflecting increased physician education and skills, improvements in medical technology, and, notably, the earlier termination of pregnancy. Legal-abortion mortality between 1979 and 1985 was 0.6 death per 100,000 procedures, more than 10 times lower than the 9.1 maternal deaths per 100,000 live births between 1979 and 1986. Serious complications from legal abortion are rare. Most women who have a single abortion with vacuum aspiration experience few if any subsequent problems getting pregnant or having healthy children. Adverse emotional reactions to abortion are rare; most women experience relief and reduced depression and distress.	Legal induced abortion-related deaths are rare events. Serious complications from legal abortion are rare.

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & comment
SAFETY OF SCREENING				
1	Statham H et al Serum Screening for Down's Syndrome: Some Women's Experiences.[General Practice] BMJ1993;307:174-76	Semistructured telephone interviews and correspondence with 20 women After a positive screening result (4) Negative amniocentesis results (8) Termination of a pregnancy with a confirmed abnormality (8).	All women were made anxious by their positive screening test, no matter how they were told. The women's experiences suggested that medical staff were unclear about the implications of screening tests and how to interpret risk. Even after receipt of negative amniocentesis results some women remained anxious. Staff did not always recognise women's concerns while awaiting amniocentesis results.	8-Poor The way in which serum screening is being implemented does not always meet the needs of women with positive results. When screening tests are introduced policies should be adopted to ensure appropriate support for participants.
2	Roelofsen et al. Women's opinion on the offer and use of maternal serum screening. Prenatal Diagnosis 1993; 13:741-7	Opinions and experiences concerning maternal serum screening of two groups of women were studied: (A) Women who were not eligible for prenatal diagnosis (B) women for whom prenatal diagnosis was available because of advanced maternal age.	80% serum screened, of whom >70% would have accepted amniocentesis if screened positive. 81% would have screening again in future. 32% had test for reassurance 26% because it is the obvious thing to do 65% not aware of drawbacks 60% satisfied with info	Serum screening is often seen as a means of reassurance and many women are not aware of the possible drawbacks.
3	Keenan KL et al Low level of maternal serum alpha-fetoprotein: its associated anxiety and the effects of genetic counseling.	Anxiety measured using State Trait Anxiety Inventory in 52 with low AFP & in 25 controls	The mean level of anxiety for the control group was 36, significantly lower than either the precounseling or postcounseling level of the group with low maternal serum alpha-fetoprotein screening results (p less than .05). The study shows that genetic counseling does significantly reduce their anxiety level	Counselling reduces anxiety in women with low AFP

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & comment
	Am J Obstet & Gynecol 1991;164:54-6			
4	Marteau TM et al The psychological effects of false-positive results in prenatal screening for fetal abnormality: a prospective study. Prenatal Diagnosis 1992; 12:205-14	346 women with initial negative result 26 women with initial positive result	Receiving an abnormal result was associated with high levels of anxiety which were reflected in increased worry about the baby's health and a more negative attitude towards the pregnancy and the baby. Those women who went on to have amniocentesis were less worried about their baby's health in the third trimester and also less anxious post-partum than those who did not have amniocentesis.	Abnormal results was associated with high levels of anxiety. A normal amniocentesis result was reassuring
5	Marteau TM et al Psychological effects of having amniocentesis: are these due to the procedure, the risk or the behaviour?. Journal of Psychosomatic Research. 1992;36:395-402	54 had amniocentesis 9 did not.	At the time of the procedure, those having amniocentesis were significantly more anxious, less certain about the baby's health, and held more negative attitudes towards the baby than women who did not undergo amniocentesis. For women undergoing amniocentesis there was a positive association between perceived risk of having an abnormal baby and anxiety. After the baby's birth, women who had undergone amniocentesis held less positive attitudes to the baby and were significantly more worried about the baby's health. The differences between the groups after the birth seem more likely to reflect pre-existing attitudinal differences between the two groups, than the effects of amniocentesis.	Anxiety surrounding amniocentesis is related both to the procedure and to the perceived likelihood of an abnormal result.
6	Santalahti et al Women's experiences of prenatal serum screening.	45 women with positive screening result 46 control women	The positive screening result and wait for the final results negatively affected the emotional well-being of most of the women, and 6 were still worried after receiving final reassuring results.	The significant negative psychosocial effects of serum screening should be

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	Grade & comment
	Birth 1996; 23:101-7		Of the 46 control women, 17 felt some worry or fear regarding abnormality in their fetus.	taken into account by caregivers when deciding whether and how to institutionalize these tests as part of antenatal care.

EVIDENCE TABLE: COST

No	Author, Title, Journal, Year	Study design, sample size, follow up	Outcomes & characteristic	
1	Bassett K et al. Triple marker screening in British Columbia: current practice, future options. BC Office of Health Technology Assessment. July 2000	HTA report	An appraisal, derived in part from primary data from children in British Columbia with Down's syndrome, estimated that, in 1997 dollars, the excess cost of health care for a person born with Down's syndrome is C$350,000.	Health cost of caring for Down's syndrome children is very high
2	Screening for Down's syndrome. U.S. Preventive Services Task Force, Gulidelines from Clinical Preventive Services, (Second Edition) 1996	Technical report	Based on 1988 cross-sectional data, the U.S.Preventive Services Task Force in 1996, estimated the lifetime economic costs of Down's syndrome to be US$410,000.	Health cost of caring for Down's syndrome children is very high
3	Wald NJ et al. Antenatal maternal serum screening for Down's syndrome: results of a demonstration project. BMJ 1992:305: 391-4	Prospective trial using a statistical model for predicting Down's syndrome risk on the basis of the three analytes and maternal age and a risk cut-off of 1:250 Sample size 12,603	The theoretical cost for each Down's syndrome birth avoided would be 28,000 pounds. The actual cost for each birth avoided was 38,000pounds. Life time costs of care in 1987 was 120,000 pounds per case of Down's syndrome	3-Fair Screening is cost-effective. However the most important reason for screening is not financial; it is the avoidance of handicap and of distress to the

				families
4	Wald NJ et al Maternal screening for Down's Syndrome in early pregnancy BMJ 1998; 297:883-7	Retrospective study of stored samples in 77 pregnancies associated with Down's Syndrome and 385 unaffected controls	It is the most effective way to screen	

EVIDENCE TABLE : LEGAL MORAL ETHICAL AND RELIGIOUS ISSUE

No	Author, Title, Journal, Years	Study design, sample size, follow up	Outcomes & Characteristic	Grade & Comment
1	Stranc LC Chorionic villus sampling and amniocentesis for prenatal diagnosis Lancet 1997, 349: 711-14	Seminar paper	Ethical issues include questions like who would benefit from prenatal screening? Is it carried out for parents to allow them to make informed decisions, for physicians to provide optimal care avoid legal liability, for society to minimize the burden of suffering or health care costs, or for the baby to be provided with a 'good start' in life	
2	Bassett K et al Triple marker screening in British Columbia: current practice, future option British Columbia Office of Health Technology Assessment 2000 July	HTA report	A central goal of prenatal counselling is 'informed choice'. Supporters of prenatal screening may argue that it provides women and couples 'choice' and thus, some degree of control over their pregnancy and reproduction. However, this 'choice' is affected by many social circumstances – some women may feel pressured into accepting prenatal screening by their doctors who feel that this is in their best interest. Other women may undergo testing at the insistence of their husbands or other family members. Consequently, many women may be going through a screening programme about which they know very little (Bassett K et al, 2000). Considered from the disability perspective, disability groups argue that prenatal screening, diagnosis and selective termination of pregnancies have a eugenic potential that constitutes a threat to disabled people as it	

			may foster a more negative image of disability and lead to increased discrimination against the disabled	
3	El-Hashemite N The Islamic View in genetic preventive procedures The Lancet 1997 July; 350(9072)	Correspondance	In the United Kingdom, Muslims do not attend genetic counselling and preventive programmes due to their religious and cultural beliefs that they not wish to interfere with natural events A study by Roberts and colleagues in Birmingham, UK, about the representation of ethnic minorities at genetic clinics, showed that family doctors refer Pakistani families with genetic disorders less frequently to genetic clinics than families from other ethnic groups, since doctors believe that Pakistani families do not wish to attend prevention programmes due to their religious beliefs The termination of pregnancy remains a contentious issue in many societies. The Muslim juriconsults (Sunni and She'at) agree that if genetic tests proved definitely that a fetus is affected by serious disease that will keep him disabled after birth, then abortion is permissible and lawful provided the termination of pregnancy is carried out before the time of breathing of the soul, that is, 120 days of gestation	
4	Salihu HM Genetic counseling among Muslims; questions remain unanswered Lancet 1997 Oct; 350 (9083)	Correspondence	In the United Kingdom, Muslims do not attend genetic counselling and preventive programmes due to their religious and cultural beliefs that they not wish to interfere with natural events In Cameroon, where Muslims constitute 25-30% of the population, .it has been said that it is difficult to offer comprehensive guidance for many Muslim couples since abortion is forbidden in Islam there have been concerns raised that while this may be acceptable to literate Muslims, much of the Muslim population depend on the local Imams (Muslim leaders), most of whom are strongly against termination of life, even before breathing of the soul	

NEURAL TUBE DEFECTS

1. BACKGROUND

Screening for neural tube defects provides an excellent 'point of entry' for an initiative aiming to reduce the burden of congenital malformations for many reasons. These malformations are so obvious that they are rarely missed in newborns and reliable audit data can be obtained, provided they are registered. In addition, many of the anomalies may be prevented by peri-conceptional dietary folate supplementation for women. Apart from this, most cases can be detected antenatally by ultrasound scanning, permitting the option of termination of pregnancy. Finally, genetic approaches developed for these disorders can be extended across the full spectrum of congenital malformations.

2. INTRODUCTION

Neural Tube Defects (NTDs) such as spina bifida, anencephaly and encephalocele are the most common severe congenital abnormalities detected at birth, with a rate of more than 2 per 1 000 births in some areas. The incidence of NTD varies geographically, with a racial variation as well, and in many countries seems to be falling over time. In fact, the birth prevalence of NTDs has fallen by 84 – 95 % as a result of a screening program, (Rodriguez L, 1997; Cuckle HS 1994; Chan A, 1993). In addition, screening could also reduce severe spina bifida in live born babies by 38.8% (Baker PA, 1981).

Currently in Malaysia there is no routine screening programme for neural tube defects. Statistics for hospitals under the Ministry of Health Malaysia for 1999 documented 225 cases of neural tube defects out of 1055 cases of lethal congenital malformation However, most pregnant women would have had at least one ultrasound during her pregnancy, where the majority of anencephalic fetuses and other congenital abnormalities are successfully detected.

3. TECHNICAL FEATURES

There are two main well-established methods of screening – measurement of maternal serum alpha-fetoprotein (MSAFP) and high-resolution ultrasound scanning (Thornton JG 1993). Fetal neural tube defects may be associated with elevated level of alpha-fetoprotein in maternal serum, maternal plasma and amniotic fluid (Clarke PC 1977).

Alpha-fetoprotein (AFP) is a glycoprotein similar to albumin, whose function is unknown. The results of serum AFP cut-off level expressed as multiples of the median (MoM) for unaffected pregnancies at that gestation. Serum AFP level expressed as MoM appears to be more convenient than percentile, easier to derive and more stable (Wald N, 1977). The clinical evaluation of a single anitbody radioimmunoassay assay reaffirms the value of the screening (Needleman, 1980). A recent study has indicated the automated fluorometric immunoassay was more precise and thus improves detection rate (Wald NJ, 2000). In elevated MSAFP, a cutoff of

2.5 multiples of the gestational age-adjusted normal median is used and this is a widely accepted standard (Evan MI 1987).

The observation of elevated maternal serum alpha fetoproteins (MSAFP) had led to demands for universal screening of maternal blood at a stage of pregnancy when termination is still possible (Clarke PC, 1977). Routine obstetrical care now includes screening for elevated levels of maternal serum alpha-fetoprotein in many areas (Nadel AS, 1990). In Spain, for example, MSAFP screening has been identified as being the best tool to identify and reduce NTD (Candenas M, 1995). It is also used as an adjunctive tool to identify high-risk pregnancies and adverse fetal outcomes (Milunski A, 1989). In the Netherlands, the triple test includes MSAFP and satisfies some of the absolute criteria of genetic screening of the Dutch Health Council (Wildhagen MF 1996).

Single antibody radio-immuno assay reaffirms the value of the screening (Needleman SB, 1980). However, a recent study by Wald NJ (2000) found that the automated fluorometric immunoassay was more precise and improves detection and reduces the false positive rate.

In ultrasound examination, there are many features seen that are characteristic of NTD. The "lemon sign" (mild hydrocephalus, frontal flattening) is present in 98% of fetuses at gestation less than 24 weeks, and in 13% of those more than. 24 weeks. Cerebellar abnormalities are present in 95% of fetuses irrespective of gestation. However, at gestation less than 24 weeks, the predominant cerebellar sign is the "banana sign" (72%) - posterior displacement of the cerebellum due to Arnold-chiari malformation - whereas at gestation more than 24 weeks, the characteristic sign is cerebellar "absence" (81%) (van den Hof MC, 1990). Apart from this, microcephaly is present in 69% and ventriculomegaly in 63% of fetuses before 24 weeks of gestation. Ultrasound cranial markers are extremely valuable to ultrasonographers at all skill levels for they not only improve the accuracy of diagnosis, but also allow a tentative diagnosis to be made 1 to 2 weeks before the detection of the spinal lesion (Thornton JG 1993; Thiagarajah S 1990).

4. METHODOLOGY

An electronic search using MEDLINE, HealthSTAR database using various keywords, age limit from 19-44 years and year limits from 1990 to 2001and English publication only. In addition, local published report and studies and other important references were obtained from various sources

5. RESULTS

5.1. Effectiveness of Screening

It has been suggested that a screening program should only be established where there is excellent interdisciplinary support among the obstetrician, laboratory, clinical geneticist, ultrasonographer and an identified programme coordinator (Milunski A, 1984). In addition, pre-screening clinical history is very important, since otherwise, it may limit the testing option for risk cases (Walpole IR 1993). Apart from this, a health visitor must be available to trace women at risk and arrange their further investigation, while a public education campaign may also need

to be introduced. (Sadovnic AD 1983, Baker PA 1981, Grace HJ 1981, Burton BK 1988, Layde PM 1979, Tosi LL 1987).

The British Columbia Office of Heath Technology Assessment indicates that the College of Physicians and Surgeons recommended not to offer the guidelines because of concern that without education an efficient referral system, to recommend the test would be premature and do more harm than good. (The British Columbia Office of Heath Technology Assessment, 1998).

5.1.1 Maternal serum alpha-fetoprotein.

MSAFP screening has been described as being the best tool to identify and reduce NTD in countries like Spain (Candenas M, 1995). It has also been used as an adjunctive tool to identify high-risk pregnancy and adverse fetal outcome (Milunski A, 1989).

Timing of screening

The level of MSAFP rises with increasing gestational age, so that the best time for it to be measured is during the second trimester at 16 – 18 weeks (Wald NJ, 1977; Brock DJ, 1975). The sensitivity of AFP screening in population-based antenatal screening for neural defects was between 70-91% (Macri JN, 1982; Persson PH, 1983). Screening of anencephaly by AFP can only detect 92% of cases (Chan A, 1995). A comparative study demonstrated that while both fetal anencephaly and exomphalos are associated with increased levels of MSAFP at 10-14 weeks (first trimester), but the sensitivity is only 30% with false positive of 5%. This supports that screening for NTD at 10-14 weeks gestation unlikely to be beneficial. (Sebire NJ 1997)

However, a study in the UK showed that only 25.7% of pregnant mothers were booked at the optimum time for MSAFP i.e. between 16 – 23 weeks (Clarke PC 1977). A retrospective study of 148 cases of NTD in England and Wales done by Williamson P et al (1997) found that the anomaly was not detected in 66% cases, of whom 20 were not offered the screening due to late booking.

Accuracy of maternal serum alpha-fetoprotein testing

A retrospective analysis of 700 samples from both normal pregnancies and pregnancies complicated by NTDs showed 90% of anencephaly, 70 % of spina bifida and 3% of normal pregnancies had a level of MSAFP 2.4 times the normal median equivalent to 97th centile (Tovey KC, 1979). Approximately 5 to 10% of patients who undergo MSAFP screening with elevated MSAFP level are found to have normal fetuses. In South Africa a pilot study of 3153 samples revealed 4.7% of women had elevated level due to incorrect data, apart from a small percentage of twins, premature labour, intra-uterine growth retardation and spontaneous abortion (Grace HJ, 1981). Another study found that only 15 out of 452 women with abnormal MSAFP had an affected fetus (Burton BK, 1988). The sensitivity of serum alpha-fetoprotein in population-based antenatal screening for neural tube defects was between 70 – 91 % (Macri JN 1982, Persson PH 1983). Screening of anencephaly by MSAFP detect 92 % of the cases (Chan A 1995).

It has been said that congenital anomalies identified by an alpha-fetoprotein-screening program are small in number. This is because there is considerable overlap between maternal serum alpha-fetoprotein values in normal pregnancies and in pregnancies complicated by an anomaly, so that the likelihood of a patient with an elevated level of MSAFP carrying an affected fetus is

low (Nadel AS, 1990). The false positive rate ranged between 0.2 – 30 % (Sebire, 1997; Cuckle H, 1990; Wald N, 1981) depending on the population as well as other factors such as multiple pregnancies, timing of screening and the level of expertise. At 16 – 18 weeks, 3% unaffected fetus had AFP level 2.5 times the median. The number of unaffected pregnancies was reduced by one third if those with borderline MSAFP results were re-tested (Wald N, 1977). However, if MSAFP were combined with real time ultrasound, the accuracy of diagnosis is very high with only one fetus out of 50 being incorrectly diagnosed and terminated (Bell WO, 1996). Two case reports of false positive diagnosis of spina bifida with elevated MSAFP who underwent termination of pregnancy after an ultrasound examination and declining amniocentesis, were found to be triploidy (Johnson DD 1997).

Maternal weight is an important factor accounting for false positives in antenatal screening for NTDs. The mean MSAFP level for women weighing less than 45 kg was 68 % higher than the mean level in women weighing 85 kg or more (Wald N 1981). A higher sensitivity and specificity of MSAFP can be achieved with absolute control of gestational age, weight, diabetes etc. It was found that the percentage of women with raised MSAFP was less when gestational age was estimated by ultrasound cephalometry as compared to menstrual age. The detection rate was 88 % with 0.9 % false positive was achieved with routine biparietal diameter measurement and AFP cut-off level of 3.0 MoM (Wald N 1982). Methods of weight adjustment like log-linear regression or reciprocal-linear are both satisfactory, but it is preferred that adjustmenst be made base don local population data (Watt HC, 1998; Kennedy DM, 1999; Neveux LM 1996). The physiologic basis for the decreased MSAFP in diabetic pregnancy is still obscure but the concentration of MSAFP may be corrected for the observed decrease, the value for unaffected cases measured in pregnant women with insulin-dependent diabetes being 0.78 MoM in one study (Henriques CU, 1993).

It has been found that second trimester dried blood screening for neural tube defect achieves a screening efficiency comparable to serum-based protocol with distinct advantages over conventional method of blood collection (Macri JN 1996).

If the AFP level is high, the serum screen is repeated. If both serum levels are high after adjustment of weight and race, the patient is offered an ultrasound examination. If the ultrasonogram does not explain the deviation from normal such as incorrect estimation of gestational age, multiple gestation, fetal death, or definite congenital anomaly, then a genetic amniocentesis is offered (Nadel AS 1990, Evans MI 1987).

5.1.2 Ultrasound

Accuracy

Many centers have moved away from MSAFP screening for detecting open neural tube defects and now rely instead on real-time routine ultrasound screening (Tyrrell S, 1988). However, in South Australia it as found that if MSAFP screening was not in place, the level of sensitivity of ultrasound screening for spina bifida would have been 62% compared to 76% when the program was actually in existence (Chan A, 1995).

The sensitivity of ultrasound for anencephaly is 100% even in a low risk population (Chan A, 1995; Clarke PC, 1977; Morrow RJ, 1991; Robert CJ, 1983; Thornton JG, 1993), and ranges from 50 – 96 % for open spina bifida (Platt LD, 1992; Clarke PC, 1977; Thornton JG, 1993;

Lindfors KK, 1987; Robert CJ, 1983). The overall detection rate is in the range of 40 - 60 % (Persson, 1983; Chan A, 1995). The reliability of ultrasound depends on the level of risk involved. Many studies have shown that the use of ultrasound in a population that has been previously screened, or in a high risk group of pregnant women such as those with a previously affected pregnancy, are both highly sensitive and specific. The sensitivity of high-resolution ultrasound for screening of NTDs in such high risk groups range from 89 – 97% with a specificity of 100% (Platt LD, 1992; Chan A, 1995; Morrow RJ, 1991). Ultrasound screening achieved 100% sensitivity and specificity in the cases referred to tertiary or teaching hospital for confirmation by other ultrasonographers (Chan A, 1995). However, it has been noted that it is unlikely that that 80% detection rate of spina bifida by diagnostic ultrasound can be sustained all patients (Robert CJ, 1983).

Timing
Prenatal ultrasound performed at 18 – 20 weeks showed maximum sensitivity, the mean gestational age being 18.3 weeks (Lennon CA 1999).

The ultrasonographic detection rate of 91.1% for open spina bifida is significant enough to warrant amniocentesis for patients with elevated MSAFP. Patients deserve to make an informed decision about whether or not to proceed with amniocentesis. Thus, the detection of NTDs by ultrasound remains inadequate as a single entity to identify all cases of spina bifida. (Platt LD 1992). However, another study showed that the use of ultrasound in previously screened population is highly sensitive and specific in the majority of cases obviating the need for amniocentesis (Morrow RJ 1991)

5.1.3. Amniocentesis
Amniotic Fluid AFP screening at between 13 to 15 weeks of gestation has been found to be sensitive for open NTDs (Crandall BF, 1995). In South Australia, it has been found if MSAFP is not in place, the level of sensitivity of amniocentesis screening for spina bifida was 62% compared to 76% when the actual program was in existence (Chan A, 1995). Amniocentesis can be performed if there is moderately elevated MSAFP with positive ultrasound or highly elevated MSAFP with negative ultrasound. However, amniocentesis is not important if the level of MSAFP is moderately elevated and ultrasound is negative (Ennever FK, 1995). In addition, it has been pointed out ultrasonography were perfectly accurate in diagnosing neural-tube defects, there would be little point in performing amniocentesis if no fetal abnormality had been identified by sonography (Nadel AS, 1990).

5.1.4 Other Screening Modalities
The role of unconjugated estriol in the screening program for NTDs is limited. A combination of abnormally elevated MSAFP and low estriol is highly predictive of NTDs in particular cases of anencephaly (Yaron Y, 1998).

Imaging with echo-planer magnetic resonance was used to determine the abnormality diagnosed by ultrasound (Baker PN, 1994). In certain CNS abnormalities detected by ultrasound, the diagnosis may change after the usage of MRI (Levine D 1999). Maternal serum CA-125 has been proven ineffective, since it increases significantly in cases of fetal abnormalities associated with hydramnios (Sapir O, 1999). Another study on biochemical tests other than triple markers, showed some relationship between the NTDs and phenylalanine and tyrosine (Legge M, 1995).

A study on Inhibin A level found that it was not significantly altered in cases of open NTDs (Lambert-Masserlian GM, 2000).

Thus, It can be conclude that there is sufficient evidence to support the use of MSAFP and ultrasound as being effective to detect neural tube defect, but there is insufficient evidence to support other modalities of screening.

5.2 Safety of Screening

Amniocentesis can accurately diagnose open spina bifida, but, however, it carries with it the risk of causing abortion and pregnancy loss in patients with normal fetus. There is a higher pregnancy loss associated with elevated MSAFP levels as compared to those with normal MSAFP, the loss rate being between 0.5 –2.1% and can be increased to as much as eight-fold (Tyrrell S, 1988; Morrow RJ, 1991).

Spontaneous abortion occurred in 0.8% of pregnancies subjected to ultrasound alone, compared to 2.1% after amniocentesis. However, in women taking sodium valproate, MSAFP levels are not sufficiently reliable, so that ultrasound and amniocentesis are unavoidable (Omtzigt JG, 1992). Apart from this, false positive MSAFP screening results may cause severe emotional effects (Tyrrell S, 1988).

No data was available on the other possible complications secondary to amniocentesis such as infection, fetal trauma or bleeding.

It can be concluded that amniocentesis may cause spontaneous abortion.

5.3 Cost Implications

The cost-benefit analysis of screening programmes is based on the sensitivity of maternal serum alpha-fetoprotein, diagnostic accuracy of ultrasound, the cost of amniocentesis as well as the lifetime costs of spina bifida or other non-lethal neural tube defects.

The cost benefit analyses of establishing and running a mass serum AFP service in many developed and underdeveloped countries were in favor of screening. A study in British Columbia with an incidence of NTD of 1.55 / 1 000 births, suggests that a screening programmed would be beneficial. It has been found that targeted ultrasound, in patients with elevated MSAFP results in potential annual savings, even without amniocentesis, after considering moral and medico-legal implications (Vintzileos AM, 1999)

It has been said that a screening program for NTDs in low risk mothers may result in substantial savings in direct health care costs if the screening protocol is followed rigorously and efficiently. It could be easily integrated into the existing routine of antenatal care requiring only some extra time for an explanation on the objective of screening (Brock DJ, 1978).

Thus, it can be concluded that there is sufficient evidence that there are cost benefits from a screening programme .

5.4 **Psychological , Social, Ethical & Legal implications**

5.4.1 Psychological Implications

There is a need to provide information, emotional reassurance on the prevention of fetal and maternal harm (Press N et al, 1997). It has been suggested that the psychological effect of the screening of NTD has to be carefully evaluated and managed in order to avoid undesirable complications (Fearn J, 1982). It has also been found that even giving a negative result to patients does not provide reassurance without effective communication about the test (Kidd J, 1993).

There has been a low uptake of the test, confirming that the provision of early information about the test is likely to improve uptake and decrease anxiety (Kyle D, 1988). In Oxford, the uptake-screening test was 72% (Wald NJ 1979). Another study concluded that anxiety related to the possibility of being screened positive probably causes decreased participation. Hence, reducing false positives by improvements in the screening tool would alleviate maternal anxiety and probably lead to a more stable participation (Raush DN, 2000). In Toronto, a cohort study on the psychological outcome following MSAFP suggested that the screening did not cause serious psychological effect to the women (Goel V, 1998).

It can be thus concluded that there is sufficient evidence on the need for providing adequate information and emotional reassurance to alleviate maternal anxiety

5.4.2 Social implications

It has been said that while patients tend to associate screening with abortion, the clinic personnel see it as a routine practice. That is perhaps one of the reasons that the rapid adoption of MSAFP screening has not been publicly debated in any widespread fashion. (Browner CH, 1997). It has also been found that after reading the educational material on this issue, women were more hesitant than medically orientated women to employ abortion as a mean of intervention. (Howard SB, 1991). Another study has found that the provider characteristics and insurance status of patients influence the women's uptake of MSAFP (Jenkin-Woelk LD, 1998).

Gynaecologists and neurologists were found to be less in agreement compared to physicians when the pregnancy is normal, but, there was high acceptance if fetus was malformed or had lethal genetic disease (Casanueva, 1997). Considering attitudes among medical personnel, 38 % of midwives did not feel that termination of pregnancies with fetuses with Down's syndrome was justified. (Khalid L, 1994). In the US, for example, it was found that women who electively terminated NTDs affected pregnancies were disproportionately white, highly educated and use folic acid more often (Velie EM, 1996). Most doctors actually agree that the screening program be made available to all pregnant women although they personally are against abortion and their views vary according to their specialty and position (Hemminki E, 2000).

It has been found that the religious affiliation of women undergoing screening influences their outlook on fetuses with neural tube defect and Down's syndrome as well (Bell M, 2000).

5.4.3. Ethical implications

Accurate prenatal diagnosis plays a major role in the care of the fetus and in the counselling of parents prenatally for pediatric neurological problems. The termination of affected fetuses is

allowed in many countries depending on the severity of the anomaly. In addition, some of the abnormalities can be treated in-utero or managed after the babies have been delivered. Counseling must emphasize that the screening is voluntary and the need for further evaluation if positive, including amniocentesis (ACOG Educational Bulletin 1996).

5.4.4 Legal implications

There was no literature on the implications of refusal to be screened. In addition there were very few studies on the medico-legal aspect of screening neural tube defects. However, there have been instances where legal action was brought against health care providers for wrongful birth by parent of a child with neural tube defects or other abnormalities, who had allegedly failed to provide appropriate counselling or information (Browner CH, 1997).

6. CONCLUSION

There is sufficient evidence to support the use of triple serum marker combined with ultrasound in second trimester, but there are major ethical and religious issues among the Muslim population

7. RECOMMENDATIONS

To the ethical and religious issues surrounding prenatal screening, invasive diagnostic procedures and termination of pregnancies, routine antenatal maternal serum screening for neural tube defects is not recommended. However, screening should be made available to women who request for the test.

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60. Tyrrell S Howel D et. *al Should maternal AFP estimation be carried out in centers where ultrasound screening is routine*? Am J Obstet Gynaecol 1988; 158: 1092-9

61. Van den Hof MC. et al. *Evaluation of lemon and banana signs in one hundred thirty fetuses with open spina bifida*. Am J Obstet 7 Gynaecol 1990; 162: 322 – 7.

62. Velie EM, Shaw GM. *Impact of prenatal diagnosis and elective termination on prevalence and risk estimates of neural tube defects in California,* 1989-1991. Am J Epidemiol 1996; 144: 473-9.

63. Vintzileos AM. et al. *Cost-benefit analysis of targeted ultrasonography for prenatal detection of spina bifida in-patient with elevated concentration of second-trimester maternal serum alpha-fetoprotein.* Am J Obstet & Gynaecol 1999; 180: 1227 – 33.

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65. Wald N, Cuckle H, et al. *Effect of estimating gestational age by ultrasound cephalometry on the specificity of AFP screening for NTDs.* Br J Obstet Gynaecol 1982; 89: 1050-3.

66. Wald N, Cuckle H, et al. *MSAFP measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K Collaborative study on AFP in relation to NTDs*. Lancet 1977; 1(8026): 1323 – 32.

67. Wald N, Cuckle H, et al. *The effect of maternal weight on serum alpha-fetoprotein level*. Br. J. Obstet Gynaecol 1981; 88(11): 1094 – 6.

68. Wald NJ, Hackshaw AK, George LM. *Assay precision of serum AFP for NTDs and Down's syndrome.* J Med Screen 2000; 7(2): 74-7.

69. Walpole IR, Philips J, et al. *The limitation of referral level fetal ultrasound examination in the detection of spina bifida in Western Australia, 1990 – 1991*. Med J Aust 1993; 159(7): 441 – 4.

70. Watt HC, Wald NJ. *Alternative methods of maternal weight adjustment in maternal serum screening for Down's syndrome and NTDs*. Pren Diagn 1998; 18(8): 842-5.

71. Wildhagen MF, Christiaens GC, Habbema JD. *Serum screening in pregnantwomen for Down's syndrome and open NTDs; testing against the Health Council criteria for genetic screening.* Ned Tijdschr Geneeskd 1996; 140(2): 85-9

72. Williamson P, Alberman E, et al. *Antecedent circumstances surrounding neural tube defect births in 1990-1991.* Bri J Obstet Gynaecol 1997; 104:

73. Yaron Y, Hamby DD, et al. *Combination of elevated MSAFP and low estriol is highly predictive of anencephaly.* Am J Med Genet 1998; 75: 297 – 9.

EVIDENCE TABLE –NTD – TECHNICAL FEATURES

No	Author, Title, Journal, Year, Vol. Number, page	Study design, Sample size, Follow-up	Characteristics & Outcomes	Comment & Grade
1.	Candenas M, Villa R, et al. MSAFP screening for neural tube defects. Report of a program with more than 30000 screened pregnancies. Acta Obstet Gynaecol Scand 1995 Apr; 74(4): 266-9.	Non-controlled clinical series 30,000 pregnancies in Asturias, in the north of Spain. MSAFP screening has been set up in 1987in order to make possible prenatal diagnosis of NTDs. Prevalence of children born with NTDs.	MSAFP screening is the best tool to identify and reduce NTD in the Spain.	Level 8 – Poor
2.	Clarke PC, Gordon YB. et al. Screening for fetal NTDs by MSAFP determination. Br J Obstet Gynaecol 1977 Aug; 84(8): 568-73.	Non-controlled clinical series & descriptive study A total of 5,539 pregnant women at 3 maternity units in London were screened for NTD by MSAFP.	Of the women tested before 23 weeks, 300 had elevated MSAFP and 14 of them had fetal abnormalities. Of the women examined before 23 weeks, half of twin and 16.7% of spontaneous abortion had elevated MSAFP.	Level 8 – Poor
3.	Evan MI, Belsky RL, et al. Establishment of a collaborative university-commercial MSAFP screening program: A model for tertiary center outreach. Am J Obstet Gynaecol, 1987; 156: 1441-9	Review of the collaborative university-commercial laboratory statewide MSAFP program in order to overcome the problem due to expansion of the availability of tertiary services beyond major medical centers. Review was done after 4 months of the program in the two large university-based MSAFP screening programs in Michigan.	The detection frequency of neural tube defects has been comparable with other program (1 in 1690) Thee aneuploidy were found in the amniotic fluid of 118 women less than 30 years who underwent amniocentesis for low MSAFP. Conclusion: 1. The establishment of university-commercial may provide a successful model for tertiary center outreach 2. Data suggest that the high risk group can be identified from low risk women 3. Low MSAFP may likely to be more important	Level 8 - Poor
4.	Milunsky A, Jick SS, et al.	Non-controlled clinical series	Relative Risk - High MSAFP NTDs = 224, major congenital defect = 4.7, fetal	Level 8 – Poor.

No	Author, Title, Journal, Year, Vol. Number, page	Study design, Sample size, Follow-up	Characteristics & Outcomes	Comment & Grade
	Predictive value, relative risks and overall benefits of high and low MSAFP in singleton pregnancies. Am J Obstet Gynaecol 1989 Aug; 161(2) 291-7	13 486 singleton pregnancies interviewed at the time of screening (15-20 weeks) 3.9% had high MSAFP 3.4% had low MSAFP	death = 8.1, NND = 4.7, LBW = 4.0, newborn complication = 3.6, oligohydramnious = 3.4, abruption = 3.0, PE = 2.3. Relative Risk - Low MSAFP Chromosomal defect = 11.6, fetal death = 3.3. MSAFP screening provides an important adjunctive tool for the identification of high-risk pregnancy and adverse fetal outcome.	
5.	Nadel AS, Jennifer KG, et al. Absence of need for amniocentesis in patients with elevated levels of MSAFP and normal sonographic examination. N Engl J Med 1990; 323: 557-61.	Retrospective review of ultrasound findings in 51 fetuses with spina bifida, encephalocele, gastroschisis or omphalocele that were delivered or aborted at a single hospital to estimate the sensitivity for these diagnoses. All cases undergone prenatal sonography between 16 – 24 weeks\ Calculation of the probability of an affected fetus with a given level of MSAFP and normal ultrasonogram.	The four types of anomalies were correctly identified in all cases – sensitivity was 100 % 95% confidence interval, 94 to 100 %) Using the lower limit of the confidence interval – the probability of an affected fetus ranges from 0.01 to 0.15% for MSAFP levels ranging from 2.0 to 3.5 times the median respectively. So this level of risk is less than the reported risk of abortion due tom the procedure of amniocentesis – some women may decide not to proceed to amniocentesis if normal sonogram .	Level 8 - Poor
6.	Needleman SB, Goldstein AI, et al. Clinical evaluation of a single antibody RIA assay for AFP. J Reprod Med 1980 Sep; 25(3): 101-7.	Non-controlled prospective descriptive study of 1049 MSAFP and 1211 AFAFP collected during 14 – 20 weeks of gestation in 5 laboratories. Dates were confirmed by ultrasound.	Among 1049 subjects (MSAFP)– 25 infants were born with NTDs, 1 fetal demise and 1023 were normal infants. False positive rate = 0.29% and false negative rate = 0.38%. Among 1211 subjects (AFAFP) – 23 infants were born with NTDs. False negative rate for AFAFP = 0.25% (close lesion). No false positive. This study reaffirms the value of screening for NTDs by means of serum AFP RIA. It negates the argument that serum screening would	Level 8 – Poor.

No	Author, Title, Journal, Year, Vol. Number, page	Study design, Sample size, Follow-up	Characteristics & Outcomes	Comment & Grade
			promote excessive amniocentesis.	
7.	Wald N, Cuckle H, et al. MSAFP measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on AFP in relation to NTDs. Lancet 1977 Jun 25; 1(8026): 1323-32	Non-controlled clinical series, descriptive study-multi-center. 19 centers collaborated in a study to determine the efficiency of MSAFP measurement as a method of screening for NTDs between 10 – 24 weeks of pregnancy. 18 684 singleton & 163 twin without NTDs 301 singleton with NTDs.	The best time to detect spina bifida is at 16 – 18 weeks. Serum AFP cut-off level expressed as MoM may be more convenient than percentile – easier to derive & more stable. Proportion of unaffected pregnancies with high MSAFP will vary depending on precision of AFP and gestation. At 16 – 18 weeks: 88% of anencephaly, 79% open spina bifida & 3% unaffected fetus had AFP level 2.5 times the median. The number of unaffected pregnancy can be reduced by 1/3 if borderline MSAFP retested. In UK, women with MSAFP > 2.5 MoM at 16-18 weeks will have 1 in 20 chance of having spina bifida & 1 in 10 of having NTDs. Result indicates that screening pregnant women by MSAFP is an effective method of selecting women for U/S & amniocentesis.	Level 8 – Poor.
8.	Wald NJ, Hackshaw AK, George LM. Assay precision of serum AFP for NTDs and Down's Syndrome. J Med Screen 2000; 7(2): 74-7.	Maternal serum samples from 470 singleton pregnancies collected between 15 – 22 weeks. Samples had been assayed using an in house radioimmunoassay and using an automated fluorometric immunoassay	Outcome: Detection and false positive rate. Comparatively, current AFP assay are more precise. Using 2.5 MOM, the false positive rate in screening for anencephaly and open spina bifida was 0.8& with new assay compared to 2% using previous assay. Improvement in the precision of maternal serum measurement have led to small but useful improvements in screening for open NTDs and Down's syndrome.	Level 8 - Poor
9.	Thiagarajah S. et al. Early Diagnosis of Spina Bifida:	Cohort study From August 1, 1986 through July 31,	Open spina bifida was diagnosed in 24 of 44 total cases of various types of NTD.	Level 6 - Fair

No	Author, Title, Journal, Year, Vol. Number, page	Study design, Sample size, Follow-up	Characteristics & Outcomes	Comment & Grade
	The Value of Cranial Ultrasound Markers. Obstet Gynaecol 76:54, 1990	1989, 4317 patients were referred to Prenatal Diagnosis and Treatment Center at University of Virginia for genetic evaluation because of maternal age, family history, abnormal MSAFP and suspicious ultrasound findings. Evaluation for NTD was done by experience ultrasonographers: 1. Lemon sign 2. BPD 3. Banana sign 4. Lateral ventricle. 5. Spine Gestational age was based on menstrual dating and femur / humerus length.	The lemon sign and cerebellar abnormalities (banana sign) were identified in all 16 fetuses in which the diagnosis of spina bifida was made between 16 – 24 weeks. Out of this, in 4 fetuses, the lemon sign and cerebellar abnormalities were detected 1-2 weeks before the appearance of spinal lesion. Microcephaly was present in 69% and ventriculomegaly in 63% before 24 weeks. Lemon sign became less reliable after 24 weeks – only 25% whereas the ventriculomegaly increase in frequency to 75%. Microcephaly and cerebellar abnormalities were present in all cases diagnosed after 24 weeks This study suggests that the use of these cranial markers is extremely valuable to ultrasonographers at all skill levels. These signs not only improve the accuracy of diagnosis but also allowed a tentative diagnosis to be made 1-2 weeks before detection of spinal lesion. Thus findings indicate that the cranial ultrasound marker is extremely reliable for the early diagnosis of spina bifida	
10.	Thornton JG; Onwude JL. Prenatal diagnosis Progress in Obstet and Gynaecol, 1993; 10:13-29	Review on prenatal diagnosis of structural and chromosomal abnormality – NTDs, Down's syndrome, cystic fibrosis, thalassemia, tay-sachs disease and sickle cell anemia.	Some screening tests are usually applied to relatively large low risk population. The rate of NTDs is varied according to geographical area and can be as high as 2 per 1000 birth. A positive family history is an important factor. Two method of screening-MSAFP and high resolution ultrasound. MSAFP usually is measured between 15 and 17	Level 9 - Poor

No	Author, Title, Journal, Year, Vol. Number, page	Study design, Sample size, Follow-up	Characteristics & Outcomes	Comment & Grade
			weeks and value related to gestation. Results are expressed as MoM and usually fetus with NTDs have an average of 4 MoM – Amniocentesis should be done if the level 2.0 – 2.5 MoM. If the incidence is 1 in 1000 – MSAFP is 2.5 Mom – the false positive rate will be 3% and detection rate is about 80% The sensitivity of ultrasound for anencephaly is 100% and for open spina bifida is between 60 – 96%. To achieve maximum sensitivity – ultrasound should be done at 18-20 weeks. A combination of MSAFP to alert an ultrasonographer combined with detailed scanning may achieve the best result of detection and should be practiced but has not been tried in practice yet	
11.	Van den Hof MC. et al. Evaluation of the lemon and banana signs in one hundred thirty fetuses with open spina bifida. Am J Obstet and Gynaecol 1990; 162: 322 – 7.	Non-randomized controlled prospective trial. During a 3-year period 1986-1988, 1561 patients at high risk for NTD were referred for a detailed ultrasound – personal or family H/O NTD, ingestion of antifolate drugs, raised MSAFP and suspected fetal NTD from routine ultrasound. After confirm gestational age, ultrasonographers were instructed to examine the fetal head before examining the spine. The incidence and diagnostic accuracy of the lemon and cerebellar ultrasonographic markers as well as head size and ventriculomegaly, were evaluated and the relationship with gestational age was	Open spina bifida was diagnosed in 130 patients. In 5 fetuses with open spina bifida at < 24 weeks, the parent elected to continue the pregnancy. The lemon sign was present in 98% of fetuses at < 24 weeks gestation but in only 13% of those at > 24 weeks gestation. Cerebellar abnormalities were present in 95% of fetuses irrespective of gestation. However the cerebellar sign at < 24 weeks gestation was predominantly the banana sign (72%) whereas at gestation > 24 weeks it was cerebellar “absence”(81%). Both growth retardation and cerebral ventriculomegaly significantly worsened with gestation while the head circumference remained disproportionately small through gestation.	Level 4 –Fair

No	Author, Title, Journal, Year, Vol. Number, page	Study design, Sample size, Follow-up	Characteristics & Outcomes	Comment & Grade
		determined.	Presence data suggest that in patients with normal ultrasound findings of fetal spine, cranium and cerebellum – the chance of undetected spinal lesion must be extremely low – therefore amniocentesis is unnecessary. On this basis, a new approach is proposed for the investigation of patients at high risk for fetal open spina bifida	
13	Wildhagen MF, Christiaens GC, et al. Serum screening for Down's Syndrome and NTDs; testing against the Health Council criteria for genetic screening Ned Tijdschr Geneeskd 1996 Jan 13; 140(2): 85-9	Theoretical evaluation To check whether serum screening for Down's Syndrome and Neural Tube Defect satisfies the criteria set by the Committee Genetic screening of Dutch Health Council.	Population serum screening satisfies some of the absolute criteria but programmes-specific conditions should be realized in an actual screening programme. Psychological and community consequences of such a screening have not yet been investigated in the Dutch population.	Level 9 - Poor

EVIDENCE TABLE –NTD- EFFECTIVENESS OF SCREENING

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
1.	Baker PA Cost benefit analysis of screening spina bifida N Z Med J 1981 Jun 10; 93(685): 386-9	Non-control clinical series Cost benefit analysis of establishing and running a mass serum AFP screening service in Auckland Hospital.	Population of 14 066 pregnant women - 8017 women screened for NTDs Screening could reduce severe spina bifida in live born babies by 38.8 %	Level 8-Poor
2.	Burton BK. Elevated MSAFP:	Expert Committee / Consensus.	MSAFP screening should be offered to all as a routine component of prenatal care. This technology not only provides an efficient and cost-effective method of screening for NTDs that is applicable	Level 9 – Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	interpretation & follow-up. Clin Obstet Gynaecol 1988 Jun; 31(2): 293 – 305.		to all pregnancies but also provide physician with important information relevant to other complication of pregnancy.	
3.	Grace HJ Prenatal Screening for neural tube defects in South Africa. An assessment 1991.	Expert committees - An assessment using natal population as a model. 122000 pregnancies annually 38725 might be screen with Enzyme-linked immunosorbent assay. 98 expected fetuses with NTDs – 32 should be detected (16 spina bifida).	Cost of caring an affected child – R 145 660 Cost of screening – R 87 825 - R 2 744 per fetus with NTDs - R 5 498 per fetus with spina bifida - Financially the introduction of mass screening of MSAFP is justified, but before this can be done adequate ultrasound facilities must be provided and permanent health visitor must be available to trace women at risk and arrange their further investigation. A public education campaign should also be introduced.	Level 9 - Poor
4.	Layde PM, von Allmen, Oakley GP Jr Maternal serum alpha-fetoprotein screening: a cost-benefit analysis. Am J Public Health 1979 Jun; 69(6): 566-73.	Expert Committee Prenatal screening: - MSAFP - + U/S Amniocentesis when indicated	Cohort of 100 000 pregnant women who would elect for termination of the affected fetuses The total cost of the program to screen 100 000 women was $ 2 047 780 and Economic benefit exceeded $ 4 000, 000.	Level 9 – Poor
5.	Milunsky A, Alpert E Results and benefits of a maternal serum alpha-fetoprotein screening program. JAMA 1984 Sep 21; 252(11): 1438 – 42.	Cohort study – private suburban 21 000 non-diabetic & 442 diabetic women with apparently normal pregnancy. Used 2.5 or greater MOM as normal upper limit.	249 or 1.2 % had a raised MSAFP 25 or 1.2 / 1000 birth of NTDs - 18 detected by screening - 2 detected by U/S - 3 had closed lesion - 2 normal MSAFP (anencephaly) Diabetic: 2.3% had raised MSAFP with 0.9% had NTDs. 13 raised MSAFP due to other congenital abnormalities. One in 400 had recommended amniocentesis. Detection efficiency before 24 week was 85.7% for anencephaly, 62.5% for open & closed spina bifida & 100% for encephalocele.	Level 6 – Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
			A screening program should be established only where there is linked excellent interdisciplinary support among obstetrician, laboratory, clinical geneticist, ultrasonographers, and an identified program coordinator	
6.	Sadovnick AD, Baird PA A cost- benefit analysis of a population screening programs for neural tube defect. Prenatal Diagnosis 1983 Apr-Jun; 3(2): 117 – 26	Cost-benefit analysis 39 000 births annually in British Columbia Population screening by measuring MSAFP with appropriate follow-up	Population screening for NTD is possible. The incidence of neural tube defect is 1.55 per 1000 births (0.94 per 1000 live births) o Result from cost-benefit analysis suggests that the outlined screening program would be beneficial.	Level 9 - Poor
7.	Tosi LL, Detsky AS, et al. When does mass screening for open NTDs in low risk pregnancies result in cost saving? CMAJ 1987 Feb 1; 136(3): 255-65.	Expert committees Cost savings for mass screening with MSAFP in low risk pregnancies was estimated using decision analysis model.	Screening could be expected to save $8 per pregnancy given a cost of $ 7.50 for the MSAFP and $42 507 for hospital and rehabilitation for the first 10 years of life for spina bifida. Extensive sensitivity analysis showed the saving was sensitive to the cost of MSAFP and highly sensitive to the specificity of the test. A screening program for NTDs in low risk may result in substantial saving in direct health care cost if screening protocol is followed rigorously and efficiently.	Level 9 – Poor
8.	(14) Walpole IR, Phillips J, et al. The limitation of referral level fetal ultrasound examination in the detection of spina bifida in Western Australia, 1990-1991. Med J Aust 1993 Oct 4; 159(7): 441-4	Non-controlled clinical series. 47 infants born with spina bifida in 1990 and 1991 at Western Australia referral center. No universal MSAFP & performances of U/S at referral level is of variable quality. U/S screening for spina bifida	False negative in 14 out of 47 infants. 6 of 14 had relevant family or medical history. Ad-hoc fetal U/S via existing referral centers had obvious limitation in detecting spina bifida in a low risk population. MSAFP has a well documented role as 10 out of 14 missed cases by U/S would have been ascertained. The study indicates that adequate pre-screening clinical history was not sought – limiting testing option for at- risk group.	Level 8 – Poor Study emphasized the important of review of sensitivity & specificity of anatomical fetal U/S – operator & equipment dependent.

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		at > 16 weeks failed when it gave a negative result.		
MATERNAL SERUM ALPHA FETOPROTEIN				
9	Milunsky A, Jick SS, et al. Predictive value, relative risks and overall benefits of high and low MSAFP in singleton pregnancies. Am J Obstet Gynaecol 1989 Aug; 161(2) 291-7	Non-controlled clinical series 13 486 singleton pregnancies interviewed at the time of screening (15-20 weeks) 3.9% had high MSAFP 3.4% had low MSAFP	Relative Risk - High MSAFP NTDs = 224, major congenital defect = 4.7, fetal death = 8.1, NND = 4.7, LBW = 4.0, newborn complication = 3.6, oligohydramnious = 3.4, abruption = 3.0, PE = 2.3. Relative Risk - Low MSAFP Chromosomal defect = 11.6, fetal death = 3.3. MSAFP screening provides an important adjunctive tool for the identification of high-risk pregnancy and adverse fetal outcome.	Level 8 – Poor.
10	Candenas M, Villa R, et al. MSAFP screening for neural tube defects. Report of a program with more than 30000 screened pregnancies. Acta Obstet Gynaecol Scand 1995 Apr; 74(4): 266-9.	Non-controlled clinical series 30,000 pregnancies in Asturias, in the north of Spain. MSAFP screening has been set up in 1987in order to make possible prenatal diagnosis of NTDs. Prevalence of children born with NTDs.	High sensitivity and specificity of MSAFP when it is done with absolute control of gestational age, weight, diabetes, etc. Poor sensitivity second level U/S for early diagnosis of spina bifida with no bulge. Incidence of NTDs is constant in Asturias (1.5-1.6 per 1000) but declined in prevalence of children born with the defects. MSAFP screening is the best tool to identify and reduce NTD in the Spain.	Level 8 – Poor
TIMING OF SCREENING				
11	Brock D.J.H; Scrimgeour J.B, et al. Effect of gestational age on screening for neural tube defects by maternal plasma measurement. Lancet 1975 Aug; 195 – 6.	Maternal plasma or serum AFP of 62 pregnancies (women attended the antenatal clinic in Oxford and Edinburgh) which resulted in an infants with neural tube defects were measured between 8 and 22 weeks of pregnancy.	Between 13 and 22 weeks of pregnancy: 54% had AFP values greater than three times the median for unaffected pregnancies. (very high) Between before 13 weeks of pregnancy: Only 5 % had value greater than 3 times and it was not very high. Study indicates that in antenatal screening for NTDs, MSAFP should be done during second trimester.	Level 8 - Poor
12	Chan A. et al.	Non-randomized controlled	For pregnancies with NTD screened by any methods – 86%	Level 4 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	The sensitivity of ultrasound and serum alpha-fetoprotein in population-based antenatal screening for neural tube defects, South Australia 1986 – 1991. British Journal of Obstetrics and Gynaecology 1995; 102: 370 – 376	prospective trial. The sensitivity of all-antenatal screening methods for neural tube defects was determined in the 243 births and termination of pregnancy in South Australia in 1986 – 1991. After ascertainment of the cases – serum and amniotic fluid result were obtained from the only laboratory performing the test and information on ultrasound were obtained from case notifications, hospital records, and medical practitioners. In relation to comparison between the ultrasound and MSAFP, both open and close lesion were included. MSAFP was considered significantly elevated if greater that 99th centile unless the abnormal finding on ultrasound examination. Primary U/S screening – routine U/S Secondary U/S screening – performed for high risk cases Tertiary U/S screening – referral from other ultrasonographers.	sensitivity was achieved. U/S screening for anencephaly achieved 100% even in low risk compared to 92 % by MSAFP. The sensitivity of ultrasound screening increased with the level of risk in pregnancy – 60% in low risk, 89% in high risk and 100% in the cases referred for confirmation by other ultrasonographers. Ultrasound screening achieved higher sensitivity in teaching hospital – mostly tertiary screening. It has been estimated that if the MSAFP not in place, the level of sensitivity of ultrasound +/- amniocentesis screening for spina bifida is 62% compared to the actual situation of 76% with the programmed in existence. Study suggests that the serum screening programmed should continue pending a significant improvement in the sensitivity of routine ultrasound screening for spina bifida. Study also suggested that the level 2 ultrasound should be performed in all cases with an elevated MSAFP, with amniocentesis being utilized when ultrasound finding are uncertain or the whole spine cannot be visualized.	
13	Clarke PC, Gordon YB. et al. Screening for fetal NTDs by MSAFP determination.	Non-controlled clinical series & descriptive study A total of 5,539 pregnant women at 3 maternity units in	Only 25.7% of women booked at optimum time for MSAFP (16-23 weeks). Of the women tested before 23 weeks, 300 had elevated MSAFP and 14 of them had fetal abnormalities.	Level 8 – Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	Br J Obstet Gynaecol 1977 Aug; 84(8): 568-73.	London were screened for NTD by MSAFP.	Of the women examined before 23 weeks, half of twin and 16.7% of spontaneous abortion had elevated MSAFP.	
14	Persson PH; Kullander S, et al. Screening for fetal malformations using ultrasound and measurements of AFP in maternal serum. Br Med J (Clin Res Ed) 1983 Mar 5; 286(6367): 747-9.	Cohort study U/S and semi quantitative MSAFP were used to detect NTD & omphalocele in 10 147 pregnancies. Selected cases of these pregnancies underwent amniocentesis.	Screening with two independent methods found 8 out of 10 NTDs (spina bifida, anencephaly. Encephalocele, omphalocele). Routine U/S alone detected only 4 malformations & by MSAFP alone detected only 7 malformed fetuses. The results suggest that, in a low population, U/S should be combined with MSAFP in screening for NTDs. MSAFP is indispensable and combination cost is negligible.	Level 6 - Fair
15	Sebire NJ. et al. Maternal alpha-fetoprotein in fetal neural tube and abdominal wall defects at 10 to 14 weeks of gestation. British Journal of Obstetrics and Gynaecology 1997;104: 849 – 85	Cohort study Maternal serum AFP concentration was determined in 9 pregnancies with fetal anencephaly, 7 with exomphalos containing liver, 2 with spina bifida and 100 normal controls at 10 to 14 weeks gestation – Oldchurch Hospital Ramford. MSAFP was taken at the time of ultrasound before termination of abnormal pregnancies. The normal medium of MSAFP concentration for individual value was calculated according to gestational age.	In the normal group – MSAFP increased with gestation. The median AFP in the group with fetal anencephaly and exomphalos was significantly higher than in normal fetuses: P value = 0.002. The AFP of the group with spinal bifida was not statistically different with normal fetus: P = 0.31. The data from this study demonstrates that at 10 – 14 weeks of gestation both fetal anencephaly and exomphalos are associated with increase MSAFP value but only with 30% sensitivity with false positive of 5%. The finding confirm previous study that screening for NTD at 10 – 14 weeks is unlikely to be beneficial	Level 6 - Fair
16	Wald N, Cuckle H, et al.	Non-controlled clinical series, descriptive study-multi-center.	The best time to detect spina bifida is at 16 – 18 weeks. Serum AFP cut-off level expressed as MoM may be more	Level 8 – Poor.

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	MSAFP measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on AFP in relation to NTDs. Lancet 1977 Jun 25; 1(8026): 1323-32	19 centers collaborated in a study to determine the efficiency of MSAFP measurement as a method of screening for NTDs between 10 – 24 weeks of pregnancy. 18 684 singleton & 163 twin without NTDs 301 singleton with NTDs.	convenient than percentile – easier to derive & more stable. Proportion of unaffected pregnancies with high MSAFP will vary depending on precision of AFP and gestation. At 16 – 18 weeks: 88% of anencephaly, 79% open spina bifida & 3% unaffected fetus had AFP level 2.5 times the median. The number of unaffected pregnancy can be reduced by 1/3 if borderline MSAFP retested. In UK, women with MSAFP > 2.5 MoM at 16-18 weeks will have 1 in 20 chance of having spina bifida & 1 in 10 of having NTDs. Result indicates that screening pregnant women by MSAFP is an effective method of selecting women for U/S & amniocentesis.	
17	Williamson P, Alberman E, et al. Antecedent circumstances surrounding neural tube defect birth in 1990-1991 British Journal of Obstetrics and Gynaecology, 1997; 104: 51-56.	Retrospective review of antenatal case notes of 148 eligible cases of NTDs out of 308 cases reported to Office of Population Census Survey in England and Wales in 1990-1991.	To document the circumstances surrounding each affected birth and assesses the care provided. The anomaly was not detected prenatally or detected after 25 weeks in 66 %, 16% diagnosed in multiple pregnancies and 18% chose to continue pregnancy despite abnormal fetus. Of the 66% - the surrounding circumstances were screening was declined in 4%, screening was not offered due to late booking in 20%, false negative MSAFP result in 5%, false negative ultrasound in 20%, both screening give false negative result in 11% and 5% due to other reasons. The estimated sensitivity of ultrasound for anencephaly was 100% but the sensitivity of ultrasound for spina bifida was lower compared to MSAFP (70-84% Vs 84-92%) Late booking precluded the offer in a substantial proportion. The presence of multiple fetuses with one or more NTD fetuses was a serious additional complication to treatment and counseling. Ultrasound scanning was a major component but associated with lower sensitivity compared to MSAFP other than anencephaly.	Level 8 – Poor
18	Macri JN, Weiss RR.	Non-controlled clinical series & descriptive study.	Voluntary screening of MSAFP identified 20 of the 22 cases (91%).	Level 8 – Poor.

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	Prenatal serum alpha-fetoprotein screening for NTDs. Obstet Gynaecol 1982 May; 59(5): 633-9.	17 703 unselected pregnancies within Long Island, New York were screened for NTDs.	692 participants demonstrated serial elevations: 24% had underestimated gestational age and 13% had multiple gestations. 53% had amniocentesis with 5.5% detection yield. No false positive or false negative. Perinatal data of first 9300 participants also identify a correlation between rate of perinatal loss & MSAPF level.	
ACCURACY OF SERUM ALPHA FETOPROTEIN TESTING				
19	Bell WO; Nelson LH, et al. Prenatal diagnosis and pediatric neurosurgery. Pediatr Neurosurg 1996; 24(3): 134-7;discussion 138.	Non-controlled clinical series of care of fetuses and newborn and alternative for parents. 50 singleton pregnancies with various types of NTDs from Jan 1990 to June 1993.	Using the method available (MSAFP & advance in real time U/S), the accuracy of diagnosis is very high with only one fetus out of 50 being incorrectly diagnosed. Additionally, the advantages of being able to counsel the parent regarding their unborn child allow them to make an informed decision. Accurate prenatal diagnosis plays a major role in the care of the fetus and in the counseling parent prenatally for pediatric neurosurgical problem.	*Level 8 – Poor.*
20	Chan A. et al. The sensitivity of ultrasound and serum alpha-fetoprotein in population-based antenatal screening for neural tube defects, South Australia 1986 – 1991. British Journal of Obstetrics and Gynaecology 1995; 102: 370 – 376	Non-randomized controlled prospective trial. The sensitivity of all-antenatal screening methods for neural tube defects was determined in the 243 births and termination of pregnancy in South Australia in 1986 – 1991. After ascertainment of the cases – serum and amniotic fluid result were obtained from the only laboratory performing the test and information on ultrasound were obtained from case notifications, hospital records,	For pregnancies with NTD screened by any methods – 86% sensitivity was achieved. U/S screening for anencephaly achieved 100% even in low risk compared to 92 % by MSAFP. The sensitivity of ultrasound screening increased with the level of risk in pregnancy – 60% in low risk, 89% in high risk and 100% in the cases referred for confirmation by other ultrasonographers. Ultrasound screening achieved higher sensitivity in teaching hospital – mostly tertiary screening. It has been estimated that if the MSAFP not in place, the level of sensitivity of ultrasound +/- amniocentesis screening for spina bifida is 62% compared to the actual situation of 76% with the programmed in existence.	Level 4 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		and medical practitioners. In relation to comparison between the ultrasound and MSAFP, both open and close lesion were included. MSAFP was considered significantly elevated if greater that 99th centile unless the abnormal finding on ultrasound examination. Primary U/S screening – routine U/S Secondary U/S screening – performed for high risk cases Tertiary U/S screening – referral from other ultrasonographers.	Study suggests that the serum screening programmed should continue pending a significant improvement in the sensitivity of routine ultrasound screening for spina bifida. Study also suggested that the level 2 ultrasound should be performed in all cases with an elevated MSAFP, with amniocentesis being utilized when ultrasound finding are uncertain or the whole spine cannot be visualized.	
23	Cuckle H, Wald N, et al. Maternal serum alpha-fetoprotein screening for open neural tube defects in twin pregnancies. Prenatal Diagnosis 1990 eb; 10(2): 71-7. AS	Non-controlled clinical series, descriptive studies. 46 twin pregnancies with open neural tube defects (22 anencephaly & 24 open spina bifida). 169 unaffected twins. Estimation of detection rate & false positive at 16 – 18 weeks.	Conventional cut-off level of 2.5 MOM - 99 % for anencephaly - 89 % for spina bifida - 30 % false positive Detection rate at 5.0 MoM with 30% false positive - 83% for anencephaly - 39% for spina bifida	Level 8 - Poor
24	Evan MI, Belsky RL, et al. Establishment of a collaborative university-commercial MSAFP screening program: A model for tertiary center outreach. Am J Obstet Gynaecol, 1987; 156: 1441-9	Review of the collaborative university-commercial laboratory statewide MSAFP program in order to overcome the problem due to expansion of the availability of tertiary services beyond major medical centers. Review was done after 4	The detection frequency of neural tube defects has been comparable with other program (1 in 1690) Thee aneuploidy were found in the amniotic fluid of 118 women less than 30 years who underwent amniocentesis for low MSAFP. Conclusion: 1. The establishment of university-commercial may provide a successful model for tertiary center outreach	*Level 8 - Poor*

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		months of the program in the two large university-based MSAFP screening programs in Michigan.	2. Data suggest that the high risk group can be identified from low risk women 3. Low MSAFP may likely to be more important	
25	Grace HJ, Gray R, Conradie JD. Prenatal detection of neural tube defects by maternal serum alpha-fetoprotein assay S Afr Med J 1981 Aug 22; 60(8): 319-24	Report of a pilot study in which 3153 maternal serum sample received during 4-month period in South Africa Out of these, 3102 were assays using enzyme-linked immunosorbent assay and 2096 pregnancy outcomes were recorded.	On the first assay, 147 or 4.7% of women had elevated level but half of it due to incorrect dates. Other than 4 fetuses with NTDs, 6 women carried twin pregnancy, 10 had premature delivery or IUGR & 4 had spontaneous abortion as a cause of elevated level of MSAFP. Two affected fetuses were missed because it was screened after 20 weeks. Incidence in Indian was 1in 1000 and colored 1in 600. However incidence in white is higher (1/130) which probably due to sampling error	*Level 8 - Poor*
26	Henriques CU, Damm P, et al. Decreased AFP in amniotic Fluid and maternal serum in diabetic pregnancy. Obstet Gynaecol 1993 Dec; 82(6): 960-4.	Non-controlled clinical series & descriptive study. MSAFP, AFAFP or both were measured in 287 pregnant women with insulin-dependent diabetes mellitus. AFP value was correlated with HbA1C, early fetal growth delay & congenital malformed.	The MoM of AFAFP was 0.89 & MSAFP was 0.78. A statistically significant correlation of HbA1C with MSAFP but not with AFAFP. The level of AFP did not correlate with growth delay. A physiologic basis for the decreased AFAFP & MSAFP is still obscure. Screening for congenital abnormalities in diabetic pregnancies should include both mid-trimester U/S and MSAFP. Concentration of AFAFP may be corrected for the observed decrease.	Level 8 – Poor.
27	Johnson DD, Nager CW, Budorick NE. False-positive diagnosis of spina bifida in a fetus with triploidy. Obstet Gynaecol 1997; 89: 809 – 11 copy 1997 by The American College of	Case report Two case reports of patient with elevated MSAFP underwent pregnancy termination after ultrasound examination. Both cases declined amniocentesis after counseling	Case 1: o Elevated MSAFP o Ultrasound findings – lemon sign, banana sign, an effaced cisterna magna and splayed of lumbar vertebrae. Case 2: o MSAFP = 3.77 MoM at 16 weeks o Ultrasound findings – lemon sign, banana sign, effaced	Level 9 - Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	Obstetricians and Gynaecologists.		cisterna magna and splayed of lumbar vertebrae on coronal view. Both case – no spinal lesion noted after termination of pregnancy. Both were a case of 69, XXY after chromosomal analysis. Skeletal X-ray showed relative narrowing of the thoracic spine – so reliance on the cranial finding, elevated MSAFP, and over interpretation of lumbar splaying resulted in an incorrect diagnosis of neural tube defect. o This report suggest that this case underscore the difficulty of diagnosing neural tube defects in utero and demonstrate that the false-positive rate for the banana sign is not zero as been previously reported	
28	Kennedy DM; Edwards VM; Worthington DJ Evaluation of different weight correction methods for antenatal serum screening using data from two multi-centre programmes Ann Clin Biochem 1999 May; 369Pt 3): 359-64	Comparing both methods of weight correction factors – Log-linear regression and reciprocal-linear regression Using data from 2 screening programmes - Using AFP and total HCG (n =129,143) - Using AFP and free beta-HCG (n = 39 982)	The reciprocal linear method fitted the data more closely but did not significantly alter the detection rate or screen positive rate for Down's syndrome and NTDs with either dataset. Both correction methods smoothed out the variability in the screen positive rate for Down's syndrome but reciprocal-linear regression was much better at reducing the variability in screen positive rate for NTD and its use is therefore worthwhile.	Level 9 - Poor
29	Macri JN, Weiss RR. Prenatal serum alpha-fetoprotein screening for NTDs. Obstet Gynaecol 1982 May; 59(5): 633-9.	Non-controlled clinical series & descriptive study. 17 703 unselected pregnancies within Long Island, New York were screened for NTDs.	Voluntary screening of MSAFP identified 20 of the 22 cases (91%). 692 participants demonstrated serial elevations: 24% had underestimated gestational age and 13% had multiple gestations. 53% had amniocentesis with 5.5% detection yield. No false positive or false negative. Perinatal data of first 9300 participants also identify a correlation	Level 8 – Poor.

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
			between rate of perinatal loss & MSAPF level.	
30	Macri JN; Anderson RW, et al Prenatal maternal dried blood screening with AFP & Free beta HCG for open NTD & Down's Syndrome. Am J Obstet Gynaecol 1996 Feb; 174(2): 566-72.	Non-controlled prospective descriptive study of 9497 dried blood specimens from patients < 35 years. Screening efficiency of second trimester dried blood screening for open NTDs & Down's Syndrome.	Positive rate for NTDs was 2.7% & for Down's Syndrome was 2.8%. All 7 cases of open NTDs were detected within the increased risk group. 6 of 8 cases of Down's Syndrome were detected. The MoM of AFP for open NTDs was 3.5 & Down's Syndrome was 0.6. The MoM of beta HCG for Down's Syndrome was 2.4. Second trimester dried blood screening for open NTDs and Down's Syndrome can achieve screening efficiency comparable to serum-based protocol with distinct advantages over conventional method of blood collection.	Level 8 – Poor
31	Nadel AS, Jennifer K. Green, et al. Absence of need for amniocentesis in patients with elevated levels of MSAFP and normal sonographic examination. N Engl J Med 1990; 323: 557-61.	Retrospective review of ultrasound findings in 51 fetuses with spina bifida, encephalocele, gastroschisis or omphalocele that were delivered or aborted at a single hospital to estimate the sensitivity for these diagnoses. All cases undergone prenatal sonography between 16 – 24 weeks. Calculation of the probability of an affected fetus with a given level of MSAFP and normal ultrasonogram	The four types of anomalies were correctly identified in all cases – sensitivity was 100 % 95% confidence interval, 94 to 100 %) Using the lower limit of the confidence interval – the probability of an affected fetus ranges from 0.01 to 0.15% for MSAFP levels ranging from 2.0 to 3.5 times the median respectively. So this level of risk is less than the reported risk of abortion due tom the procedure of amniocentesis – some women may decide not to proceed to amniocentesis if normal sonogram.	*Level 8 - Poor*
32	Neveux LM; Palomaki GE, et al. Refinements in managing maternal weight adjustment for interpreting prenatal	Study examines the relationship between maternal weight and serum levels of AFP, unconjugated Estriol and HCG in a population of 47 585 women provided with	Reciprocal-linear equation more accurately described the weight relationship for two of three analytes than the currently used log-linear equations. However the reciprocal-linear provide only minimal advantage. A more important finding is that published weight equation may not be optimal for some screening programs due to differences in	Level 8 - Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	screening results. Prenat Diagn 1996 Dec; 16(12): 1115-9.	prenatal screening for Down's Syndrome and NTDs.	the mean weight in the populations tested. Screening programs are encouraged to calculate their own weight correction formulae based on the date of their own population.	
33	Persson PH; Kullander S, et al. Screening for fetal malformations using ultrasound and measurements of AFP in maternal serum. Br Med J (Clin Res Ed) 1983 Mar 5; 286(6367): 747-9.	Cohort study U/S and semi quantitative MSAFP were used to detect NTD & omphalocele in 10 147 pregnancies. Selected cases of these pregnancies underwent amniocentesis.	Screening with two independent methods found 8 out of 10 NTDs (spina bifida, anencephaly. Encephalocele, omphalocele). Routine U/S alone detected only 4 malformations & by MSAFP alone detected only 7 malformed fetuses. The results suggest that, in a low population, U/S should be combined with MSAFP in screening for NTDs. MSAFP is indispensable and combination cost is negligible.	Level 6 - Fair
34	Sebire NJ. et al. Maternal alpha-fetoprotein in fetal neural tube and abdominal wall defects at 10 to 14 weeks of gestation. British Journal of Obstetrics and Gynaecology 1997;104: 849 – 85	Cohort study Maternal serum AFP concentration was determined in 9 pregnancies with fetal anencephaly, 7 with exomphalos containing liver, 2 with spina bifida and 100 normal controls at 10 to 14 weeks gestation – Oldchurch Hospital Ramford. MSAFP was taken at the time of ultrasound before termination of abnormal pregnancies. The normal medium of MSAFP concentration for individual value was calculated according to gestational age.	In the normal group – MSAFP increased with gestation. The median AFP in the group with fetal anencephaly and exomphalos was significantly higher than in normal fetuses: P value = 0.002. The AFP of the group with spinal bifida was not statistically different with normal fetus: P = 0.31. The data from this study demonstrates that at 10 – 14 weeks of gestation both fetal anencephaly and exomphalos are associated with increase MSAFP value but only with 30% sensitivity with false positive of 5%. The finding confirm previous study that screening for NTD at 10 – 14 weeks is unlikely to be beneficial	Level 6 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
35	Tovey KC, Gerson M Cut-off levels for MSAFP in the diagnosis of NTDs: validation of the use of multiples of the normal median. Br J Obstet Gynaecol 1979 Jul;(7): 507-15.	Retrospective analysis of 700 plasma sample from normal pregnancies and 60 from pregnancies complicated by NTDs measured for MSAFP at 15 – 20 weeks by a standard radio immunoassay technique	90 % of women with fetuses affected by anencephaly, 70% by spina bifida & 3% normal pregnancies had plasma AFP level at or above 2.4 times the normal median for each gestation. It was estimated that 2.1, 2.4 and 3.1 times the normal median maternal plasma AFP were equivalent to the 95th, 97th and 99th centile respectively.	*Level 8 - Poor*
36	Wald N, Cuckle H, et al. The effect of maternal weight on maternal serum alpha-fetoprotein levels. Br. J Obstet Gynaecol 1981 Nov; 88(11): 1094-6.	Cohort study 902 singleton pregnancies. Level of mean MSAFP at 15 and 20 weeks related to maternal weight.	The mean MSAFP level for women weighing less than 45 kg was 68% higher than the mean level of women weighing 85 kg or more. (Significant). Maternal weight was found to be an important factor, which could account for false positive AFP results in an antenatal screening for NTDs. Policy of adjusting MSAFP value according to maternal weight among borderline positive cases could reduce the number of diagnostic amniocentesis. False positive rate of 902 pregnancies of 2.5 MoM would have been reduced from 2.8% to 2.0%	Level 6 - Fair
37	Wald N, Cuckle H, et al. MSAFP measurement in antenatal screening for anencephaly and spina bifida in early pregnancy. Report of U.K. collaborative study on AFP in relation to NTDs.	Non-controlled clinical series, descriptive study-multi-center. 19 centers collaborated in a study to determine the efficiency of MSAFP measurement as a method of screening for NTDs between	The best time to detect spina bifida is at 16 – 18 weeks. Serum AFP cut-off level expressed as MoM may be more convenient than percentile – easier to derive & more stable. Proportion of unaffected pregnancies with high MSAFP will vary depending on precision of AFP and gestation. At 16 – 18 weeks: 88% of anencephaly, 79% open spina bifida & 3% unaffected fetus had AFP level 2.5 times the median. The number of unaffected pregnancy can be reduced by 1/3 if	Level 8 – Poor.

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	Lancet 1977 Jun 25; 1(8026): 1323-32	10 – 24 weeks of pregnancy. 18 684 singleton & 163 twin without NTDs 301 singleton with NTDs.	borderline MSAFP retested. In UK, women with MSAFP > 2.5 MoM at 16-18 weeks will have 1 in 20 chance of having spina bifida & 1 in 10 of having NTDs. Result indicates that screening pregnant women by MSAFP is an effective method of selecting women for U/S & amniocentesis.	
38	Wald N, Cuckle, et al. Effect of estimating gestational age by U/S cephalometry on the specificity of AFP screening for NTDs. Br J Obstet Gynaecol 1982 Dec; 89(12): 1050-3	Non-controlled clinical series & descriptive study. 1268 women had MSAFP between 15 and 22 weeks & routine U/S at first ANC.	The percentage of women with raised MSAFP was less when gestational age was estimated by BPD compared to first day of LMP – 1.8% Vs 2.3% with 2.5 MoM. Different U/S policies and effect on AFP – the best was routine BPD used together with higher cut-off level of AFP. Detection rate was 88% & false positive only 0.9% with 3.0 MoM. Routine BPD better than scanning only with raised MSAFP or doubtful gestational age	Level 8 – Poor.
39	Watt HC, Wald NJ. Alternative methods of maternal weight adjustment in maternal serum screening for Down's syndrome and NTDs. Prenat Diagn 1998 Aug; 18(8): 842-5.	Two methods of weight adjustment were compared in a data set of 8905 singleton pregnancies without Down's syndrome and NTDs.	1. Weight adjustment based on linear relationship between the marker (MOM) and maternal weight on a linear scale. 2. Weight adjustment based on linear relationship between marker concentration and the reciprocal of the maternal weight. Both were satisfactory neither had an obvious advantage over the other.	Level 8 – Poor
40	Burton BK. Elevated MSAFP: interpretation & follow-up. Clin Obstet Gynaecol 1988 Jun; 31(2): 293 – 305.	Expert Committee / Consensus.	MSAFP screening should be offered to all as a routine component of prenatal care. This technology not only provides an efficient and cost-effective method of screening for NTDs that is applicable to all pregnancies but also provide physician with important information relevant to other complication of pregnancy.	Level 9 – Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
ACCURACY OF ULTRASOUND				
1.	Chan A. et al. The sensitivity of ultrasound and serum alpha-fetoprotein in population-based antenatal screening for neural tube defects, South Australia 1986 – 1991. British Journal of Obstetrics and Gynaecology 1995; 102: 370 – 376	Non-randomized controlled prospective trial. The sensitivity of all-antenatal screening methods for neural tube defects was determined in the 243 births and termination of pregnancy in South Australia in 1986 – 1991. After ascertainment of the cases – serum and amniotic fluid result were obtained from the only laboratory performing the test and information on ultrasound were obtained from case notifications, hospital records, and medical practitioners. In relation to comparison between the ultrasound and MSAFP, both open and close lesion were included. MSAFP was considered significantly elevated if greater that 99th centile unless the abnormal finding on ultrasound examination. Primary U/S screening – routine U/S Secondary U/S screening – performed for high risk cases Tertiary U/S screening – referral from other ultrasonographers.	For pregnancies with NTD screened by any methods – 86% sensitivity was achieved. U/S screening for anencephaly achieved 100% even in low risk compared to 92 % by MSAFP. The sensitivity of ultrasound screening increased with the level of risk in pregnancy – 60% in low risk, 89% in high risk and 100% in the cases referred for confirmation by other ultrasonographers. Ultrasound screening achieved higher sensitivity in teaching hospital – mostly tertiary screening. It has been estimated that if the MSAFP not in place, the level of sensitivity of ultrasound +/- amniocentesis screening for spina bifida is 62% compared to the actual situation of 76% with the programmed in existence. Study suggests that the serum screening programmed should continue pending a significant improvement in the sensitivity of routine ultrasound screening for spina bifida. Study also suggested that the level 2 ultrasound should be performed in all cases with an elevated MSAFP, with amniocentesis being utilized when ultrasound finding are uncertain or the whole spine cannot be visualized.	Level 4 - Fair
2.	Clarke PC, Gordon YB, et al	Non-controlled clinical series	Of the women tested before 23 weeks, 300 had elevated MSAFP and 14 had abnormalities (12 NTDs & 2 alimentary tract	Level 8 – Poor.

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	Screening for fetal neural tube defects by maternal plasma alpha-fetoprotein determination. Br J Obstet Gynaecol 1977 Aug; 84(8): 568-73 AS	5539 pregnant patients at 3 maternity units in the city & Hackney District of London were screened for NTDs (MSAFP)	abnormalities) Half of those with twin had elevated MSAFP. 16.7% of spontaneous abortion had elevated MSAFP	
3.	Lindfors KK, McGahan JP, et al. Midtrimester screening for open NTDS: correlations of sonography with amniocentesis results. AJR Am J Roentgenol 1987 Jul; 149(1): 141-5.	Non-controlled clinical series & descriptive study 70 Midtrimester pregnancies with elevated MSAFP referred for sonography and amniocentesis.	Total of 8 NTDs. 1. 2 anencephaly detected by U/S. 2. 6 cases of spina bifida were detected by amniocentesis (elevated AFAFP and acetylcholinesterase) 3. 3 of them seen on ultrasonography All pregnancies were terminated and diagnosis confirmed on pathologic examination.	Level 8 - Poor
4.	Morrow RJ, Mc Nay MB, Whittle MJ. Ultrasound Detection of Neural Tube Defect in Patients With Elevated Maternal Serum Alpha-Fetoprotein. Obstet Gynaecol 78: 1055, 1991.	Cohort study The reliability of ultrasound for detecting neural tube defects in 905 women with elevated MSAFP was assessed in Queen Mother's Hospital, Glasgow from January 1, 1985, to December 31, 1989. No amniocentesis was performed after February 1987. Perinatal pathologist examined aborted fetuses.	Among 905 pregnancies, 49 neural tubes defects were correctly diagnosed by ultrasound alone but failed to identify one NTD - SENSIVITY of 98% but SPECIFICITY of 100%. Positive predictive value was 100% and negative predictive value was 99.9%. In detecting all anomalies, there was 86% sensitivity and 100% specificity – abdominal wall defect, chromosome abnormality, urinary tract abnormality and cardiac abnormality. All anencephaly fetuses were identified before screening with MSAFP. Spontaneous abortion occurred in 0.8% of pregnancies subjected to ultrasound alone and 2.1% after amniocentesis. MSAFP screening is a relatively low cost test. Study showed the	Level 6 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		Diagnosis of NTD at delivery were verified by a neonatal pediatrician Normal babies were confirmed by delivering obstetric staffs	use of ultrasound in previously screened population is both highly sensitive and specific, and thus in the majority of cases obviates the needs for amniocentesis. Study suggests that regional centers with improved ultrasound equipment and training should be able to rely on ultrasound as the main technique for the diagnosis of spina bifida when MSAFP is elevated	
5.	Persson PH; Kullander S, et al. Screening for fetal malformations using ultrasound and measurements of AFP in maternal serum. Br Med J (Clin Res Ed) 1983 Mar 5; 286(6367): 747-9.	Cohort study U/S and semi quantitative MSAFP were used to detect NTD & omphalocele in 10 147 pregnancies. Selected cases of these pregnancies underwent amniocentesis.	Screening with two independent methods found 8 out of 10 NTDs (spina bifida, anencephaly. Encephalocele, omphalocele). Routine U/S alone detected only 4 malformations & by MSAFP alone detected only 7 malformed fetuses. The results suggest that, in a low population, U/S should be combined with MSAFP in screening for NTDs. MSAFP is indispensable and combination cost is negligible.	Level 6 - Fair
6.	Platt LD. et al. The California Maternal Serum Alpha-Fetoprotein Screening Program: The role of ultrasonography in the detection of spina bifida. Am J Obstet Gynaecol 1992; 166: 1328-9.	Non-controlled clinical series – descriptive study. Between January and June 1990, > 640,000 women were screened in the California MSAFP Screening Program and the cases with elevated serum AFP underwent an ultrasound examination by a sonographer who met the criteria defined by Genetic Disease Branch. When appropriate, amniocentesis was offered – if amniotic AFP elevated, an acetylcholinesterase was ascertained.	161 cases of spina bifida were identified and only 148 cases or 91.1% could be said to be detected by ultrasound before amniocentesis. Another 10 cases were only identified by ultrasound after amniocenteses yield positive result. Three cases were not recognized until birth – amniocentesis not done. Two additional cases of closed spina bifida were not identified – low level of MSAFP and normal amniotic AFP. The ultrasonographic detection rate of 91.9% for open spina bifida is significantly enough below 100% to warrant amniocentesis for patients with elevated MSAFP. Patient deserves to make an informed decision about whether to proceed with amniocentesis.	Level 8 - Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
			Study suggests that the detection of NTD with ultrasound remain inadequate as a single entity to identify all cases of open spina bifida.	
7.	Roberts CJ, Evan KT, et al Diagnostic effectiveness of ultrasound in detection of neural tube defect The Lancet, November 5, 1983: 1068 – 1069	Case series 2509 pregnant women were investigated between 1977 to 1983 in two studies (1977-1980 and 1980 – 1983) to assess the effectiveness of diagnostic ultrasound to detect NTDs. The diagnostic ultrasound was done for women judged to be at risk based on high MSAFP, family history and congenital malformed.	The detection rate for anencephaly was 100%. In both study. However the detection rate for spina bifida improved from 33% to 80% and specificity rose from 96% to 99%. The false positive rate dropped from 57% to 9% and false negative rate fell from 1% to 0.3%. It is unlikely that the effectiveness of detection of spina bifida of 80% could be sustained if diagnostic ultrasound to be offered to all patient because in this study all cases were already selected. Consideration will need to be given whether detection rate for spina bifida will be worth the additional cost incurred in providing diagnostic ultrasound to every patient.	Level 8 - Poor
8.	Thornton JG; Onwude JL. Prenatal diagnosis Progress in Obstet and Gynaecol, 1993; 10:13-29	Review on prenatal diagnosis of structural and chromosomal abnormality – NTDs, Down's syndrome, cystic fibrosis, thalassemia, tay-sachs disease and sickle cell anemia.	Some screening tests are usually applied to relatively large low risk population. The rate of NTDs is varied according to geographical area and can be as high as 2 per 1000 birth. A positive family history is an important factor. Two method of screening- MSAFP and high resolution ultrasound. MSAFP usually is measured between 15 and 17 weeks and value related to gestation. Results are expressed as MoM and usually fetus with NTDs have an average of 4 MoM – Amniocentesis should be done if the level 2.0 – 2.5 MoM. If the incidence is 1 in 1000 – MSAFP is 2.5 Mom – the false positive rate will be 3% and detection rate is about 80% The sensitivity of ultrasound for anencephaly is 100% and for open spina bifida is between 60 – 96%. To achieve maximum sensitivity – ultrasound should be done at 18-20 weeks. A combination of MSAFP to alert an ultrasonographer combined with detailed scanning may achieve the best result of detection and should be practiced but has not been tried in practice yet	Level 9 – Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
9	Tyrrell S; Howel D, et al. Should maternal AFP estimation be carried out in centers where ultrasound screening is routine? Am J Obstet Gynaecol 1988; 158: 1092-9. SAFE SCREEN	Re-examined the policy of relying exclusively on ultrasound examination for the detection of spinal lesion. Calculations are made on the basis of published assessments of the performances of these tests and the assumption about the test independence. Test conclusions against a wide variety of test performance values in a detailed sensitivity analysis.	Final decision to carry out maternal serum AFP testing is a value judgement, but this decision should be based on realistic numeric estimates of the potential benefits and hazards of this procedure.	*Level 9 - Poor*
TIMING				
9.	Lennon CA, Gray DL. Sensitivity and Specificity of Ultrasound for the Detection of Neural Tube and Ventral Wall Defects in a High-Risk Population. Obstet Gynaecol 1999;94:562-6 copy 1999 by American College of Obstetricians and Gynaecologists	Non-randomized controlled prospective Trial. From August 1988 to July 1997, 2257 women at high risk for fetuses with an open neural tube defect were evaluated – 1757 due to elevated MSAFP and 516 had family history of NTD. Sonologist obstetrician-geneticists did ultrasound. Amniocentesis was done in 204 patient after counseling due to various reasons such as inadequate visualization, very high MSAFP, patients at risk of chromosomal abnormality or requested by them. Postnatal follow-up	The mean gestational age for prenatal ultrasound and amniocenteses were 18.3 weeks In 2053 patients who has sonography alone detected 55 cases of NTD and another 11 cases detected after ultrasound and amniocentesis. Sensitivity of ultrasonography was 97% reflecting 2 of the cases with suspicious finding require amniocentesis for confirmation. Specificity was 100%. There was 100 % sensitivity and specificity of ultrasound examination for detecting ventral wall defect. Study suggest that when used by experience operators, prenatal sonography is sensitive and specific for the diagnosis of NTD and ventral wall defect in a targeted at-risk population	Level 4 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		information was obtained in 100% of cases. The diagnostic accuracy of sonography was compared with that of amniocentesis and sonography.		
10	Platt LD. et al. The California Maternal Serum Alpha-Fetoprotein Screening Program: The role of ultrasonography in the detection of spina bifida. Am J Obstet Gynaecol 1992; 166: 1328-9.	Non-controlled clinical series – descriptive study. Between January and June 1990, > 640,000 women were screened in the California MSAFP Screening Program and the cases with elevated serum AFP underwent an ultrasound examination by a sonographer who met the criteria defined by Genetic Disease Branch. When appropriate, amniocentesis was offered – if amniotic AFP elevated, an acetylcholinesterase was ascertained.	161 cases of spina bifida were identified and only 148 cases or 91.1% could be said to be detected by ultrasound before amniocentesis. Another 10 cases were only identified by ultrasound after amniocenteses yield positive result. Three cases were not recognized until birth – amniocentesis not done. Two additional cases of closed spina bifida were not identified – low level of MSAFP and normal amniotic AFP. The ultrasonographic detection rate of 91.9% for open spina bifida is significantly enough below 100% to warrant amniocentesis for patients with elevated MSAFP. Patient deserves to make an informed decision about whether to proceed with amniocentesis. Study suggests that the detection of NTD with ultrasound remain inadequate as a single entity to identify all cases of open spina bifida.	Level 8 - Poor
11	Morrow RJ, Mc Nay MB, Whittle MJ. Ultrasound Detection of Neural Tube Defect in Patients With Elevated Maternal Serum Alpha-Fetoprotein.	Cohort study The reliability of ultrasound for detecting neural tube defects in 905 women with elevated MSAFP was assessed	Among 905 pregnancies, 49 neural tubes defects were correctly diagnosed by ultrasound alone but failed to identify one NTD - SENSIVITY of 98% but SPECIFICITY of 100%. Positive predictive value was 100% and negative predictive value was 99.9%. In detecting all anomalies, there was 86% sensitivity and 100%	Level 6 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	Obstet Gynaecol 78: 1055, 1991.	in Queen Mother's Hospital, Glasgow from January 1, 1985, to December 31, 1989. No amniocentesis was performed after February 1987. Perinatal pathologist examined aborted fetuses. Diagnosis of NTD at delivery were verified by a neonatal pediatrician Normal babies were confirmed by delivering obstetric staffs	specificity – abdominal wall defect, chromosome abnormality, urinary tract abnormality and cardiac abnormality. All anencephaly fetuses were identified before screening with MSAFP. Spontaneous abortion occurred in 0.8% of pregnancies subjected to ultrasound alone and 2.1% after amniocentesis. MSAFP screening is a relatively low cost test. Study showed the use of ultrasound in previously screened population is both highly sensitive and specific, and thus in the majority of cases obviates the needs for amniocentesis. Study suggests that regional centers with improved ultrasound equipment and training should be able to rely on ultrasound as the main technique for the diagnosis of spina bifida when MSAFP is elevated	
AMNIOCENTESIS				
1	Crandall BF, Chua C Detecting neural tube defects by amniocentesis between 11 and 15 weeks' gestation Prenatal Diagnosis 1995 Apr; 15(4): 339-43	Non-controlled clinical series, descriptive studies. 7440 amniocenteses tested between 11 and 15 weeks of gestation. 81% tested between 13 – 15 weeks. Using a cut-off > or = 2.0 MOM.	42 open NTD were identified. Detection rate: 95 % - 100% for spina bifida & anencephaly - 78% for encephalocele 2 encephalocele had AFAFP < 2.0 MOM & negative AchEs The result indicates that amniotic Fluid AFP appears to be as sensitive a test for open NTDs between 13 – 15 weeks as between 16 and 20 weeks.	Level 8 - Poor
2	Chan A. et al. The sensitivity of ultrasound and serum alpha-fetoprotein in population-based antenatal screening for neural tube defects, South Australia 1986 – 1991.	Non-randomized controlled prospective trial. The sensitivity of all-antenatal screening methods for neural tube defects was determined in the 243 births and termination of pregnancy in South Australia in 1986 –	For pregnancies with NTD screened by any methods – 86% sensitivity was achieved. U/S screening for anencephaly achieved 100% even in low risk compared to 92 % by MSAFP. The sensitivity of ultrasound screening increased with the level of risk in pregnancy – 60% in low risk, 89% in high risk and 100% in	Level 4 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	British Journal of Obstetrics and Gynaecology 1995; 102: 370 – 376	1991. After ascertainment of the cases – serum and amniotic fluid result were obtained from the only laboratory performing the test and information on ultrasound were obtained from case notifications, hospital records, and medical practitioners. In relation to comparison between the ultrasound and MSAFP, both open and close lesion were included. MSAFP was considered significantly elevated if greater that 99th centile unless the abnormal finding on ultrasound examination. Primary U/S screening – routine U/S Secondary U/S screening – performed for high risk cases Tertiary U/S screening – referral from other ultrasonographers.	the cases referred for confirmation by other ultrasonographers. Ultrasound screening achieved higher sensitivity in teaching hospital – mostly tertiary screening. It has been estimated that if the MSAFP not in place, the level of sensitivity of ultrasound +/- amniocentesis screening for spina bifida is 62% compared to the actual situation of 76% with the programmed in existence. Study suggests that the serum screening programmed should continue pending a significant improvement in the sensitivity of routine ultrasound screening for spina bifida. Study also suggested that the level 2 ultrasound should be performed in all cases with an elevated MSAFP, with amniocentesis being utilized when ultrasound finding are uncertain or the whole spine cannot be visualized.	
3	Ennever FK, Lave LB Parent preferences and prenatal testing for NTDs Epidemiology 1995 Jan; 6(1):8-16 AMNIO	Consensus/ Expert opinion Screening battery following high MSAFP -Weighing risk of false negative Vs amniocentesis fetal loss - Included risk of false positive and parent preference	With all risk included, high resolution U/S is appropriate for all women with elevated MSAFP Amniocentesis needed if: -Moderate elevated MSAFP and positive U/S -High elevated MSAFP but negative U/S Amniocentesis not needed if: _ Moderate elevated NSAFP and negative U/S - High elevated with positive U/S	*Level 9-poor*
4	Nadel AS, Jennifer KG, et al. Absence of need for	Retrospective review of ultrasound findings in 51 fetuses with spina bifida,	The four types of anomalies were correctly identified in all cases – sensitivity was 100 % 95% confidence interval, 94 to 100 %)	*Level 8 - Poor*

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	amniocentesis in patients with elevated levels of MSAFP and normal sonographic examination. N Engl J Med 1990; 323: 557-61.	encephalocele, gastroschisis or omphalocele that were delivered or aborted at a single hospital to estimate the sensitivity for these diagnoses. All cases undergone prenatal sonography between 16 – 24 weeks\ Calculation of the probability of an affected fetus with a given level of MSAFP and normal ultrasonogram.	Using the lower limit of the confidence interval – the probability of an affected fetus ranges from 0.01 to 0.15% for MSAFP levels ranging from 2.0 to 3.5 times the median respectively. So this level of risk is less than the reported risk of abortion due tom the procedure of amniocentesis – some women may decide not to proceed to amniocentesis if normal sonogram.	
OTHER SCREENING MODALITIES				
1	Baker PN. et al. A three-year follow-up of children imaged in utero with echo-planar magnetic resonance. Am J Obstet Gynaecol 1994; 170: 32 – 3.	Non-controlled clinical series – descriptive study. A 3-year follow-up study was carried out on 20 children examined in utero with echo-planar MRI when fetal compromised has been suspected. Fetuses were imaged to determine the abnormal pregnancy, congenital abnormality diagnosed by ultrasound, suspected IUGR or others. All images were obtained with a 0.5 T super conductive magnet using the modulus-blipped echo-planar sequence. All born alive infant were	o Overall there was no demonstrable increase in the occurrence of disease or disability including hearing deficit that could be related to echo-planar imaging technique. Although this series is too small to fully demonstrate the safety but it has not been possible to link any ill effect in the fetus to echo-planar imaging	Level 8 - Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		assessed as normal by midwife at delivery being tested at 8 months old – hearing distraction test and other hearing test later		
2	Lambert-Masserlian GM; Palomaki GE; Canick JA. Second trimester of maternal serum inhibin A in pregnancies affected by fetal NTDs Prenat Diagn 2000 Aug; 20(8): 680-2	Inhibin levels were measured in second trimester maternal serum samples from 28 pregnancies affected by NTDs – 12 spina bifida & 16 anencephaly. Control – 1464 singleton pregnancies.	Inhibin A level were not significantly altered in cases of open NTDs. In spina bifida = 0.96 MoM In anencephaly = 1.19 MoM. Therefore second trimester maternal serum inhibin A will not have an impact on prenatal detection of open NTDs.	Level 8 - Poor
3	Levine D; Barnes PD, et al CNS abnormalities assessed with prenatal magnetic resonance imaging. Obstet Gynaecol 1999; 94: 1011-9.	Prospective study of 89 women with 91 fetuses from 1991 to 1996 in Children Hospital, Boston, Massachusetts. Ninety-one ultrasound and MRI were done with the indication of CNS abnormality – 25 for ventriculomegaly, 16 for NTDs, 12 for arachnoid cyst, 11 for large cisterna magna and 20 for others.	MRI findings led to change diagnosis in 26 (40%) of 66 fetuses with abnormal ultrasound. There were abnormalities not detected by ultrasound such as complete agenesis of corpus callosum, cortical cleft and so on. There were case better delineated by MRI than ultrasound included one distal neural tube defect. When CNS anomalies detected or suspected by ultrasound – MRI might led to alter diagnosis and patient counseling	Level 8 - Poor
4	Legge M Mid Second Trimester organic acids in severe open neural tube defects J. Obstet. Gynaecol Vol 21,	Amniotic fluid collected from normal, anencephalic and severe open spina bifida fetuses was analyzed for up to 24 organic acids by gas liquid chromatography.	Children with spina bifida were found to have elevated urinary p-hydroxyphenylacetic acid and homovaniolic acid concentrations. To investigate amniotic fluid organic concentration at 16 weeks gestation from fetuses and those affected by severe neural tube defects.	Biochemical test other than normally used triple markers. It shows the relationship

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	No 5: 483-487: 1995		Significant increase in organic acids similar to those observed in defects of phenylalanine and tyrosine metabolisms were identified when compared with the normal fetuses. Significant differences in organic acids concentration were also identified within the two neural tube defects groups. A relationship between phenylalaninine and tyrosine metabolism, closure rate of the neural tube and folate metabolism is proposed.	between the NTD and cases of phenylalanine and tyrosine metabolic problems. Level 8 - Poor
5	Sapir O, Holcberg G, et al Maternal serum concentration of CA-125 in second trimester pregnancy complicated by congenital fetal anomalies Eur J Obstet Gynaecol Reprod Biol 1999 Dec; 87(2): 133-6	Study population (n=40) consisted of the following 4 group of patients: ⇒ 10 women with abnormal maternal serum AFP in whom no fetal anomalies were observed ⇒ 10 women in whom fetal anomalies were diagnosed in addition to abnormal MSAFP. ⇒ 10 women with fetal anomalies accompanied by hydramnios without fetal anomaly ⇒ 10 women had normal MSAFP and were diagnosed with hydramnious without fetal anomaly. The control group consisted of 10 patients who were matched for gestational age with normal MSAFP and normal ultrasonographic examination. In all 50 cases, MSAFP and maternal serum CA-125 levels were assessed. CA-125 was	To determine the value of maternal serum CA-125 concentration in pregnancies complicated by fetal anomalies with or without hydramnios. Maternal serum CA-125 levels was significantly higher in the study group than in the control group, 19.8+/- 15.9U/ml and 9.9+/- 4.0U/ml (p=0.015). The different was even greater when patient with malformed fetuses and hydramnios were compared to those with fetal anomalies and normal amount of amniotic fluid, 32.4+/- 12.7 U/ml and 7.2+/- 2.1U/ml respectively. (P=0.00005). The maternal serum CA-125 levels in patients with hydramnios but without fetal anomalies were significantly lower when compared with those of the malformed fetuses and hydramnios, 9.8+/- 2.3U/ml and 32.4+/- 12.7U/ml, respectively (p=0.002) Maternal serum CA-125 is lacking in value for screening fetal structure anomalies as a significant increase in maternal serum CA-125 levels was found only in patients with fetal anomalies accompanied by hydramnios.	Effectiveness of certain method biochemical test (CA-125) Level 8 - Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		measured using OC 125 monoclonal antibody (IMX CA-125, Abbot lab. And value of > 20U/ml was defined as abnormal.		
6	Yaron Y, Hamby DD, et al. Combination of elevated MSAFP and low estriol is highly predictive of anencephaly. Am J Med Genet 1998 Jan 23; 75(3): 297-9.	At Centre of Fetal Diagnosis & Therapy, Michigan, USA, 57 037 patients underwent maternal serum screening with MSAFP at 14 – 22 weeks. Of these, 23 415 also had uE3 measurement.	There were 63 cases of NTDs (1.1 in 1000). MSAFP > OR = 2.5 MOM was detected in 1346, 48 had NTDs. Decreased Ue3 (< or = 0.5) was detected in 1437, 17 had NTDs. The incidence of NTDs significantly higher in patients with low estriol compared to normal/high estriol. 51 patients had both increase MSAFP and low estriol, 16 had NTDs and 14 had anencephaly. Study concluded that both elevated MSAFP and low maternal estriol are predictive of NTDs but low sensitivity. The combination of abnormally elevated MSAFP and low estriol is highly predictive of NTDs in particular anencephaly.	Level 8 - Poor
SAFETY OF SCREENING				
1.	Morrow RJ, Mc Nay MB, Whittle MJ. Ultrasound Detection of Neural Tube Defect in Patients With Elevated Maternal Serum Alpha-Fetoprotein. Obstet Gynaecol 78: 1055, 1991.	Cohort study The reliability of ultrasound for detecting neural tube defects in 905 women with elevated MSAFP was assessed in Queen Mother's Hospital, Glasgow from January 1, 1985, to December 31, 1989. No amniocentesis was performed after February 1987. Perinatal pathologist examined aborted fetuses. Diagnosis of NTD at delivery were verified by a neonatal	o Among 905 pregnancies, 49 neural tubes defects were correctly diagnosed by ultrasound alone but failed to identify one NTD - SENSIVITY of 98% but SPECIFICITY of 100%. Positive predictive value was 100% and negative predictive value was 99.9%. o In detecting all anomalies, there was 86% sensitivity and 100% specificity – abdominal wall defect, chromosome abnormality, urinary tract abnormality and cardiac abnormality. o All anencephaly fetuses were identified before screening with MSAFP. o Spontaneous abortion occurred in 0.8% of pregnancies subjected to ultrasound alone and 2.1% after amniocentesis. MSAFP screening is a relatively low cost test. Study showed the use of ultrasound in previously screened population is both highly sensitive and specific, and thus in the majority of cases obviates the needs for amniocentesis.	Level 6 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		pediatrician Normal babies were confirmed by delivering obstetric staffs.	Study suggests that regional centers with improved ultrasound equipment and training should be able to rely on ultrasound as the main technique for the diagnosis of spina bifida when MSAFP is elevated	
2.	Omtzigt JG, Los FJ, et al. Prenatal diagnosis of spina bifida aperta after first-trimester valproate exposure. Prenat Diagn 1992 Nov; 12(11): 893-7.	Cohort study Ultrasonography and amniocentesis were performed at 16-18 weeks of gestation in 267 pregnancies of 237 women using anti-epileptic drugs Results were compared with MSAFP obtained prior to amniocentesis	Among 92 pregnancies with maternal sodium valproate used, 5 were terminated due to spina bifida aperta – all prenatally diagnosed by AFP determination and acetylcholinestrase in amniotic fluid. The MSAFP were raised > or = 2.5 MOM in singleton and 4.5 MOM in twin in only two of these affected fetuses. Study emphasized that MSAFP may be un reliable for prenatal screening for NTDs in women taking valproate and recommend that amniocentesis and fetal U/S should be offered directly.	*Level 6 - Fair*
3.	Tyrrell S; Howel D, et al. Should maternal AFP estimation be carried out in centers where ultrasound screening is routine? Am J Obstet Gynaecol 1988; 158: 1092-9.	Re-examined the policy of relying exclusively on ultrasound examination for the detection of spinal lesion. Calculations are made on the basis of published assessments of the performances of these tests and the assumption about the test independence. Test conclusions against a wide variety of test performance values in a detailed sensitivity analysis.	Final decision to carry out maternal serum AFP testing is a value judgement, but this decision should be based on realistic numeric estimates of the potential benefits and hazards of this procedure.	*Level 9 - Poor*

-EVIDENCE TABLE -NTD - COST

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
1.	Brock DJ, Scrimgeour JB, et al.	Non-controlled clinical series & descriptive study	13 cases of anencephaly & 7 open spina bifida were detected by MSAFP. Further 3 cases were detected by previous medical	Level 8 – Poor

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
	Maternal plasma alpha-fetoprotein screening for fetal neural tube defects. Br J Obstet Gynaecol 1978 Aug; 85(8): 575-81	Blood sample for MSAFP were measured at 15 to 20 weeks gestation from 6377 women (79% of antenatal booking).	history followed by amniocentesis. Detection rate by MSAFP was 83%. Integration of screening into the existing pattern of ANC required only minor alteration in clinic schedules. Some extra time was needed for explanation of the objective, for U/S & for amniocentesis.	
2.	Vintzileos AM. et al. Cost-benefit analysis of targeted ultrasonography for prenatal detection of spina bifida in patients with an elevated concentration of second-trimester maternal serum alpha-fetoprotein. Am J Obstet Gynaecol 1999; 180: 1227 – 33.	Non-randomized controlled prospective trial. Cost-benefit formula was based on the hypothesis that the cost of universal genetic amniocentesis in patients with an elevated concentration of MSAFP in the second trimester should be at least equal to the cost of universal targeted U/S, with amniocentesis used only for those with an abnormal ultrasound. The component of the formula includes: Diagnostic accuracy of ultrasound. the cost of amniocentesis package (US 1200), the cost of targeted ultrasound (US 300), lifetime cost of spina bifida not detected by ultrasound. (US 330 000) A graph was constructed representing the balance between the sensitivity and false positive rate of targeted U/S. The accuracy of 17 studies using the cranial sign was examined from the cost benefit point of view.	o Assuming overall prevalence of spina bifida of 1:50, 1:100,1:200 among women with elevated MSAFP, targeted ultrasonography is beneficial only if the overall sensitivities for detecting spina bifida were >88%, >76%, and 51% respectively. o All 17 studies which used cranial sign for detecting spina bifida, had accuracy compatible with economic benefits – average sensitivity = 97.3% and false positive rate = 0.5%. Currently achieved second trimester U/S accuracies are compatible with net benefit (US 44 000 Vs US 120 000) Targeted USG in patients with elevated second trimester MSAFP in the US has the potential for annual savings of approximately US 36 – 49 million and for avoiding 268 fetal deaths. Note: The required sensitivity in the cases of diabetic and MSAFP elevation more than 4 MoM is higher. Argument against the use of USG: 1. The possibility of missing chromosomal abnormalities but half of them are sex chromosome which carry good prognosis 2. May be individual ethical and moral decision of patient to have the most sensitive test. Issue of medico-legal implication and cost of missed spina bifida cases but counterbalance with fewer fetal losses by the lesser risk of lawsuits	Level 4 - Fair

No	Title, author, Journal, Year, Vol Number, page Number	Study design, Sample size, follow up	Outcomes & Characteristic	Comment & Grade
		Sensitivity analysis includes a range of prevalence of spina bifida in women with elevated MSAFP from 1:50 to 1:200 and false positive rate of 1 – 10 %.		

EVIDENCE TABLE - NTD-PSYCOHOLOGICAL, SOCIAL, ETHICAL & LEGAL IMPLICATION

No	Title, Author, Journal, Year, Vol. Number, page Number	Study design, samples size, follow-up	Outcomes & Characteristic	Comment & Grade
SOCIAL				
1.	Bell M, Stoneman Z. Reaction to prenatal testing: reflection of religiosity and attitudes toward abortion and people with disabilities. Am J Ment Retard 2000 Jan; 105(1): 1-13	Cohort study Asked individual what they would do if through prenatal testing they discovered that they or their partner were carrying an affected fetus.	Respondents to continue pregnancy: - More uncertain if the fetus is Down's syndrome than spina bifida & hemophilia. - Less certain if the fetus is spina bifida than hemophilia. Modest support to the hypothesis that negative attitudes toward people with disabilities would be associated with an increase likelihood of choosing abortion. Religious affiliation was associated with decision concerning hemophilia but church attendance concern fetuses with all 3 diagnoses.	Level 6 - Fair
2.	Casanueva E, Lisker R, et al. Attitudes of Mexican Physicians toward induce abortion Int J Gynaecol Obstet 1997 Jan; 56(1): 47-52.	Non-controlled descriptive study 193 Physician (internist, pediatrician, gynaecologist & neurologist) with no experience with genetic – Questionnaire of attitude toward induce abortion of normal fetus & various degree of abnormal fetus.	Few physician approved abortion of normal pregnancy, 6 out of 10 if the fetus was malformed and 9 of 10 in cases of lethal genetic disease. Gynaecologist & neurologist were less in agreement when pregnancy is normal. In general the physician did not have consistent answers. Agreement for abortion was influenced by religious value.	Level 8 - Poor
3.	Khalid L, Price SM, Barrow M.	Non-controlled descriptive study	188(55%) completed the questionnaire. 40.4% did	Level 8 – Poor.

No	Title, Author, Journal, Year, Vol. Number, page Number	Study design, samples size, follow-up	Outcomes & Characteristic	Comment & Grade
	The attitude of midwives to maternal screening for Down's Syndrome. Public Health 1994 Mar; 108(2): 131-6	Questionnaire to 342 Leicestershire midwife to determine the opinion and attitude toward testing for spina bifida and Down's Syndrome.	not confidence counseling for the serum screening test. 255 were not in favour of prenatal testing and 38.3% did not feel termination of Down's Syndrome was justified. If such a program is to be introduced, more attention must be paid in advance to the view and training requirement of those midwives who will be associated with the teat. It should not be assumed that ethical consideration is of minor significant.	
4.	Hemminki E, Toiviainen H, Santalahti P. View of Finnish doctors on fetal screening. Bri J Obstet Gynaecol 2000; 107:656-662.	Anonymous, questionnaire survey conducted in 1996-1997 involved 164 gynecologist, 214 pediatricians and 166 general practitioners (leading & ordinary).	Most doctors agreed that serum screening for Down's syndrome & structural abnormalities should be available for all pregnant women. Main drawback – false positive. A few doctors against abortion. Some difference views as expected due to difference specialty and position. Finnish doctors support current fetal screening, but many acknowledge resulting ethical, psychological, and social problems.	Level 8 - Poor
5.	Velie EM; Shaw GM. Impact of prenatal diagnosis and elective termination on prevalence and risk estimates of neural tube defects in California, 1989 – 1991 Am J Epidemiol 1996; 144: 473-9.	Review prevalence : - Prevalence data were ascertained from a population-based 1989-1991 cohort of California births – 664 infants/fetuses were included. Interview data from mothers of 538 ascertained cases and 539 normal cases – to examine maternal/infants characteristic.	The birth prevalence (livebirths and stillbirth only) was 48.4% of the total prevalence (including elective termination) for anencephaly and 70.2% for spina bifida. Woman, who electively terminated NTD-affected pregnancies were disproportionately white, more highly educated higher incomes and used folic acid more often. For the factors related to elective termination – risk assessment appeared biased because only livebone and stillborn were included and not all clinically recognized.	Level 8 - Poor
6.	Jenkin-Woelk LD, Baldwin LM, et al Influence of provider characteristics	Retrospective cohort study of MSAFP use in low-risk obstetrics patients of five	Patients of urban and rural obstetricians-gynaecologists were most likely to have MSAFP testing whereas urban certified midwife and rural	Level 6 – Fair

No	Title, Author, Journal, Year, Vol. Number, page Number	Study design, samples size, follow-up	Outcomes & Characteristic	Comment & Grade
	and insurance status on maternal serum AFP screening. J Am Board Fam Pract, 1998; 11(5): 357-65	provider specialty and patient insurance status in Washington state.	family physicians were least likely to have the test. Patients of certified nurse midwife were more likely to refuse testing. Medicaid-insured women were significantly less likely to have the test compared to women who use private insurance. Providers and patients did not uniformly use MSAFP screening. So all providers should ensure that their patients were adequately informed about the screening test.	
7.	Browner CH, Nancy Press . Why women say yes to prenatal diagnosis Soc. Sci. Med; 7:979-989.	Review John McKinlay's model of how medical innovation become routinized to explore the circumstances that led to widespread use of one prenatal – MSAFP to detect NTDs.	Analysis of published data suggested that strong support from provider or institutional is the best predictor of women acceptance. Data form California illustrates that the medico-legal and institutional forces affect the use of MSAFP. Data also showed how provider shapes women's understanding of the meaning and purpose of the test. These data conformed that even very ethical issue concern critic of prenatal diagnosis become obscured in the process by which the test accepted as routine.	Level 9 - Poor
PSYCOLOGICAL				
1	Browner CH, Nancy Press . Why women say yes to prenatal diagnosis Soc. Sci. Med; 7:979-989.	Review John McKinlay's model of how medical innovation become routinized to explore the circumstances that led to widespread use of one prenatal – MSAFP to detect NTDs.	Analysis of published data suggested that strong support from provider or institutional is the best predictor of women acceptance. Data form California illustrates that the medico-legal and institutional forces affect the use of MSAFP. Data also showed how provider shapes women's understanding of the meaning and purpose of the test. These data conformed that even very ethical issue concern critic of prenatal diagnosis become	Level 9 - Poor

No	Title, Author, Journal, Year, Vol. Number, page Number	Study design, samples size, follow-up	Outcomes & Characteristic	Comment & Grade
			obscured in the process by which the test accepted as routine.	
2	Fearn J, Hibbard BM, Laurence KM, Roberts A, Robinson JO. Screening for neural-tube defects and maternal anxiety. British Journal of Obstetrics & Gynaecology 1982; 89:218 - 221	Non-randomized controlled prospective study with historical control. Anxiety levels were studied using the Speilberger State-Trait Inventory test in 176 women with raised serum alpha-fetoprotein level at the time they attended central assessment clinic of University of Wales (before amniocentesis). Second assessment (after amniocentesis) was performed 2-3 weeks later in those not found to have fetus with a neural-tube defect.	• All women attending the clinic for further assessment were extremely anxious, irrespective of the source of information – statistically highly significant increase in anxiety compared with the normal primigravida. • Anxiety score 2-3 weeks after testing in the group of women who had been given a definite normal result were significantly less anxious than the group of women who was told by the obstetrician to assume normal result if they did not hear from hospital. • Women who did not require amniocentesis after reassessment had some residual anxiety in spite of verbal reassurance. The result suggest that the psychological effect of the screening on neural tube defect need to be carefully evaluated and managed in order to avoid undesirable complications	Level 5 – Fair.
3	Kyle D, Cummins C, Stuart E. Factors affecting the uptake of screening for neural tube defect. British Journal of Obstetrics and Gynaecology. (1) 1988; 95: 560 – 564.	Cohort study All 1235 patients booking at the Birmingham Maternity Hospital in the first quarter of 1984 who were sent the standard leaflet about AFP screening were also sent a questionnaire designed both to explore their attitudes and knowledge of screening for neural tube defects as well as to assess the value of the leaflet.	o Prior knowledge about spina bifida and screening test – 95% had heard about spina bifida & 73% had heard about the screening test – less percentage from medical sources. o Response to the leaflet - 98% found the leaflet very useful and 11% (low social class) said it contain nearly all-new informations. o Acceptance of screening, anxiety about leaflet and intention and behavior - The majority said that they would accept the test, and subsequently did so. This suggest that low	Level 6 - Fair

No	Title, Author, Journal, Year, Vol. Number, page Number	Study design, samples size, follow-up	Outcomes & Characteristic	Comment & Grade
		Overall, 957 or 77.5 % of the patients responded and returned the questionnaires. Percentage of respondents to specific questions were invariably high	uptake of the test is not a result of patients resistance, and the results indicate that the provision of early information about the test is likely to improve uptake and decrease anxiety o Feeling about termination – Many women did not know whether they would accept termination if they were carry a fetus with spina bifida. The religious influenced this issue. Study suggest that with the increasing clinical usefulness of the AFP screening, routine screening of pregnant women with an "opt-out" system is becoming clinically desirable, and the survey suggest that it would be acceptable to the patients •	
4	Kidd J, Cook R, Marteau TM. Is routine AFP screening in pregnancy reassuring? J Psychosom Res 1993 Oct; 37(7): 717-22	Non-randomized controlled perspective study. 309 women tested (group 1) 30 women not tested. (Group 2) Study the impact of a negative result on a routine prenatal screening for NTDs & Down's Syndrome.	21 out of 309 think that they had not been tested (group 3) 7 out of 30 thought that they had been tested (group 4) No significant differences in anxiety, certainty about the baby's health or worry about the baby's health. Result suggests that the receipt of a negative result on this screening test does not provide reassurance. The potentially reassuring effects of such test may be realized with more effective communication about the test.	Level 4 – Fair
5	Wald NJ, Cuckle HS, et al. Antenatal screening in Oxford for fetal neural tube defects. Br J Obstet Gynaecol 1979 Feb; 86(2): 91-100	Between May 1975 & 1977, 6443 antenatal patients were screening between 16 and 22 weeks for NTD by MSAFP.	Take-up screening test = 72%. 17 out of 18 (94%) with open NTD (9 out of 9 anencephaly and 8 out of 9 spina bifida) had positive tests and 17 accept termination. 245(3.8%) unaffected pregnancies had positive screening but only 1.4% had amniocentesis. About	Level 8 - Poor

No	Title, Author, Journal, Year, Vol. Number, page Number	Study design, samples size, follow-up	Outcomes & Characteristic	Comment & Grade
			50% of unaffected pregnancy with elevated MSAFP was not offered amniocentesis due to multiple pregnancies detected by U/S. The odd of having fetus with NTDs after amniocentesis was 1 in 6. Two normal pregnancies were terminated. 73 patients with elevated MSAFP except one agrees for general screening and wanted to be tested in future pregnancy.	
6	Raush DN, Lambert Messerlian GM; Carnick JA. Participation in maternal serum screening for Down's syndrome, NTDs and Trisomy 18 following screen-positive results in a previous pregnancy. West J Med 2000 Sep; 173(3): 180-3.	Databases of screening information of 108 screen-positive and 108 screen-negative for the risk of Down's syndrome and NTDs obtained from laboratory and hospital.	In age-matched comparison, screen positive women were significantly less likely to participate in maternal serum screening in their next pregnancies. In the type of screen-positive result, the effect was significant for both Down's syndrome and NTDs. The degree of risk in screen-positive did not significantly affect their participation. Study concluded that anxiety related to screen-positive probably cause decrease participation. Reducing the screen-positive rate would alleviate maternal anxiety and probably lead to more stable participation.	Level 8 – Poor
7	Goel V; Glazier R, et al Psychological outcomes following maternal serum screening: a cohort study CMAJ 1998 Sep 22; 159(6): 651-6	A prospective cohort study with baseline assessment at 15 – 18 weeks and follow-up at 24 weeks of 2020 pregnant women (84% of potential subjects) at University of Toronto, Ontario. 1. State Trait Anxiety Test 2. Epidemiological Studies Depression Scale Eligible cases – 2020 but only 1741 completed the follow-up.	No overall adverse psychological effects as a result of testing found at 24 weeks of gestation. Women with false-positive had a mean increase in anxiety state by 1.6 whereas a true-negative had a mean decrease of 1.1 and those not tested had a mean decrease of 0.4. The mean depression score increased by 0.5 in the false-positive group was unchanged in the true negative and increase by 0.2 in the non-tested group. Of the women underwent testing – 7.6 % were	Level 6 - Fair

No	Title, Author, Journal, Year, Vol. Number, page Number	Study design, samples size, follow-up	Outcomes & Characteristic	Comment & Grade
		A total of 1177 (67.6%) underwent maternal serum screening.	unsure of their result at the time of follow-u [p. Result suggested maternal screening in Ontario is not causing serious psychological effect to women.	
ETHICAL				
1	Committee on Educational Bulletins of the ACOGs. ACOG Educational Bulletin. Maternal Serum Screening. Int J Gynaecol Obstet 1996 Dec; 55(3): 229-308.	Expert Committee / Consensus	Maternal serum screening offers women the ability to increase the detection of open NTDs, Down's Syndrome & Trisomy 18. Counseling must emphasize screening is voluntary & awareness of further evaluation if positive include amniocentesis. Physician should have access to genetic service and reliable laboratories. Special attention to older Gravida. Patients received positive result from definitive test should have access to adequate counseling including support group & paediatric surgeon .	Level 9 – Poor
LEGAL				
1	Browner CH- Nancy Press,. Why women say yes to prenatal diagnosis Soc. Sci. Med; 7:979-989.	Review John McKinlay's model of how medical innovation become routinized to explore the circumstances that led to widespread use of one prenatal – MSAFP to detect NTDs.	Analysis of published data suggested that strong support from provider or institutional is the best predictor of women acceptance. Data form California illustrates that the medico-legal and institutional forces affect the use of MSAFP. Data also showed how provider shapes women's understanding of the meaning and purpose of the test. These data conformed that even very ethical issue concern critic of prenatal diagnosis become obscured in the process by which the test accepted as routine.	Level 9 - Poor

THALASSEMIA MAJOR

1. BACKGROUND

Thalassemia is a serious single gene disorder, Beta-Thalassemia being the most common. It is Mandellian recessive, so that interaction of two common beta-Thalassemia alleles will result in a transfusion- dependent disorder. Thus, the incidence of Thalassemia major increases substantially with marriages of unsuspected Thalassemia-traits individuals. However, even couples with heterozygous alpha-Thalassemia and beta-Thalassemia are at risk of developing homozygous alpha-Thalassemia (Lam YH, 1997). Carriers can be detected by conventional blood test with an accuracy of 99%. Couples at risk can be identified before they have children and offered genetic counseling, and consequently reduce the incidence of Thalassemia major. A carrier screening programme has been set up in several countries for 20 years. (Medell B, 1997).

The incidence of Thalassemia is very high, with over 30 million people carrying the defective gene. The prevalence of the Thalassemia trait ranges from 4.3% in Gaza (Sirdah M, 1998) to 8.8% in Hong Kong. The carrier frequency ranges from 3% to 17% in different populations, being more in the Mediterraneans regions and the east (incidence as high as 15%) and less in the west (incidence 1.5%). For example, over 9 000 Thalassemic children are born every year in India. In Egypt, over 1 000 of the annual 1.5 million newborns are expected to be affected with this disorder, and 4 % of immigrants from this country are carriers of hemoglobinopathies. Thalassemia has now become a global health problem, spreading through migration from its native area in the Mediterranean through Africa and Asia and is now endemic through Europe, the Americas and Australia. The frequency of beta Thalassemia heterozygosity was 4.7% (Scriver C, 1984).

Thalassemia is the commonest single gene disorder in Malaysia, affecting mostly the Malay and Chinese population, and only a small percentage of Indians (Kaur P, 1995). In 1995, it was estimated that there are probably 8 000 afflicted persons with HbE beta Thalassemia, and 8 000 with homozygous beta-Thalassemia, with 3 000 likely to be transfusion dependent. The frequency for alpha –thalassaemia is 20%, for Hb E is 3-50%, for beta–thalassaemia is 3-4%, and for Hb Constant Spring is 1-4%. Malaysian Chinese has beta-Thalassemia similar to that encountered in Chinese patient in South China (George E, 1993). However, at the present moment, there is no national policy on Thalassemia screening in Malaysia.

2. INTRODUCTION

Thalassemia results in a considerable increase in both acute and chronic morbidity and mortality. The psychosocial impact may lead to rejection of treatment and low self- esteem. These patients also suffer the complications of multiple blood transfusions and iron over-load. Borgna-Pignatti (1998), showed survival to age 20 years was 89. Even in developed countries like UK, 50% patients with beta- Thalassemia major die before the age of 35 years (Modell B, 2000).

Antenatal screening for Thalassemia-traits in pregnant mothers, with subsequent screening of spouses in positive mothers, could predict a Thalassemia major pregnancy in couples both with

Thalassemia-traits. This could be confirmed with amniocentesis or chorionic villus sampling. Theoretically, induced abortion of this affected fetus will also reduce the incidence of Thalassemia major individuals. Because of the burden in managing Thalassemia patients, extensive population screening programs for Thalassemia have been implemented in Sardinia, the Italian province of Ferrara, Cyprus, Iran and Greece. In the United Kingdom , screening for hemoglobin traits is offered to specific higher risk populations. Premarital and prenatal screening of couples is also being carried out in Cyprus, Sardinia and Iran. Cyprus and Sardinia have also undertaken programmes to educate schoolchildren and offer them voluntary screening. Various other countries such as Australia, Isfahan, Israel, Pakistan, Sri Lanka, Thailand, and the United States, have looked into the prospects of screening to prevent Thalassemia major.

The management of beta-Thalassemia in developing countries may face organisational, logistic and funding problems. A study in India of 200 families with Thalassemia showed that treatment of these children placed a significant, unavoidable and increasing demand on the public health services. Major problems have leading to premature death among affected children (Sangani B et al, 1990). Another study reported only 60% of children with documented beta-Thalassemia were monitored more or less regularly, while the rest 40% died or were lost to follow-up. While clinical results were acceptable in terms of growth, transfusion goals (pre-transfusion Hb less than or equal to 10 g/dl) were achieved in only 7% of cases, with adverse effects to transfusions proving difficult to prevent. There were inadequate funds with only 5.4% of actual costs in drugs and small equipment being covered.

Patients with Thalassemia need red blood cell transfusions 2 to 3 weekly throughout their lives. The life expectancy for most (around 80%) is 30 years, even with iron chelating agents like desferioxamine. Due to their high costs, there are only a few chelating agents available in Malaysia. Deferiprone (L1), Kelfer and Ferriprox are reserved for patients who have iron overload and where desferioxamine is contraindicated.

3. METHODOLOGY.

Electronic literature search using MEDLINE and HEALTHSTAR databases was carried out for 1989-2000, using the following keywords: *Antenatal Thalassemia screening, Haemoglobinopathy Antenatal Screening, Haemoglobinopathy Prenatal diagnosis, Thalassemia Prenatal screening, Haemoglobinopathy screening.* All studies related to the objective were appraised and graded.

4. TECHNICAL FEATURES

Diagnosis of Thalassemia

Molecular diagnosis has the benefits of speed, cost, convenience and the ability to test for multiple mutations simultaneously. For beta-Thalassemia mutations, the procedures that meet these requirements are the *amplification refractory mutation system* (ARMS) and the *reverse dot-blot* hybridization system. For alpha-Thalassemia the technique of *gap PCR* is useful for

targeting specific deletion mutations, but the *Southern blot* remains the standard diagnostic test (Old J, 1996).

4.1. MCV / Red Cell Indices

Red cell indices obtained through standard electronic cell counters provide valuable tools for preliminary screening of thalassemic traits. Generally, there is reduced mean corpuscular volume (MCV), and reduced mean corpuscular hemoglobin (MCH) with normal mean corpuscular hemoglobin concentration (MCHC). Specific cut off points for each index varies with laboratories, some focusing on both reduced MCV and MCH, others on MCV or MCH alone (Mitchell J et al, 1996) .

4.2. Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT)

NESTROFT is a rapid, reliable, and cost effective screening test for detection of Beta-Thalassemia trait in a population that does not require sophisticated equipment. It is based on the limit of hypotonicity that the red cell can withstand. A positive test is due to reduced osmotic fragility of red cells. Consequently, subjects who are NESTROFT 'positive' or 'doubtful' deserve further investigation.

4.3. Hemoglobin A2 Estimation

The hemoglobin A2 level is considered the gold standard for diagnosis of Thalassemic trait. Subjects found to be positive in preliminary screening tests by red cell indices or NESTROFT are confirmed for thalassemic carrier status by HbA2 measurement. HbA2 levels can be measured by various methods such as microcolumn chromatography, high performance liquid chromatography (HPLC) and capillary iso-electric focusing. Subjects with HbA2 levels of 3.5 % and above are considered to have a thalassemic trait. However, for those with HbA2 levels between 3.3 and 3.8 %, it is recommended that the assay be repeated to rule out a technical error while performing the assay (Kleman K & Lubin B; 1989).

4.4. Hemoglobin electrophoresis

Comparing the protocols involving universal haemoglobin electrophoresis and selective use of haemoglobin found that the sensitivity, specificity, positive predictive value and negative predictive value of a protocol with selective use of haemoglobin electrophoresis would have been 88.9%, 79.4%, 20.8% and 99.2%.

4.5. DNA Mutation Analysis.

There are various methods available to study DNA mutations such as allele specific oligonucleotide (ASO) screening, reverse dot blot, and restriction endo-nuclease allele recognition. Another recent method is amplification refractory mutation system (ARMS) technique in which specific primers against normal and mutant sequences are used. The common mutations account for about 90-93.6%. The rest could be characterized using techniques such as single strand conformation polymorphism (SSCP) and denaturing gradient gel electrophoresis (DGGE). This can be followed by sequencing using currently available automated sequencers.

4.6. Polymerase Chain Reaction (PCR).

Polymerase Chain Reaction (PCR) enables the selective amplification of the 136 base pair regions within the alpha-globin gene cluster, as a routine test for prenatal diagnosis of

homozygous alpha zero-Thalassemia. Confirmation of PCR results is by using DNA gene mapping and electrophoresis of cord blood. PCR has been found to be fast and accurate in identifying alpha-Thalassemia carriers (Galanello R et al 1998)

4.7. Other Screening Modalities

4.7.1. Southern blot analysis and anti-zeta antibody test

Southern blot analysis and the anti-zeta antibody test can detect SEA deletion in dried blood samples, even after prolonged storage, with high specificity, and 95% sensitivity. This simple, inexpensive test can screen samples collected at a distance from a central laboratory (Harada F, Chui D, 1994).

4.7.2. *Preimplantation genetic diagnosis (PGD)*

Pre-implantation genetic diagnosis (PGD) allows couples at risk of having children with Thalassemia to ensure the healthy outcome of their pregnancy. Sequential first and second PB testing of oocytes is reliable for PGD of Thalassemia and is a feasible alternative to prenatal diagnosis in high-risk populations (Kuliev A et al, 1999).

4.7.3. Haemoglobin H inclusion bodies

Melis A et al (1980) showed that Haemoglobin H (HbH) inclusion bodies was a reliable test for identification of alpha -Thalassemia trait.

4.7.4. Slot-blot immunobinding assay

This test can detect Zeta-globin chains in carriers of alpha-Thalassemia-1 and can be used in appropriate populations to identify those couples at risk of conceiving fetuses afflicted with Hb Bart's hydrops fetalis syndrome due to homozygous alpha-Thalassemia (Luo HY et al, 1988; Skogerboe K et al, 1992)

4.7.5. Reverse Dot Blot

The reverse dot blot is a rapid hydridization method to detect specific mutation of beta-globin gene. This method is the technique of choice in Egypt, and being routinely used in Italy (Rady S et al, 1997). A study in India found both partners heterozygous for codon 15 (G-A) mutation of beta-globin gene, but the fetus was normal (Christian Medical College Hospital, 1996)

4.7.6. Low pressure liquid chromatography system (LPLC)

This system provides an accurate and sensitive alternative to traditional manual chromatography and electrophoresis method, an automated sampler allowing batches from 1-99 samples to be processed with significant saving in operator time (Chambers K et al 1993)

Screening

The detection of carrier status during pregnancy provides prospective parents with the option of testing the fetus for a hemoglobinopathy. If the test is positive, they have time to discuss continuation the pregnancy and plan optimal care for their newborn. Parents appear to act on this genetic information. It has been said that about half of pregnant women with positive tests for Thalassemia refer their partners for testing and, if the father is positive, about 60% consent to

amniocentesis. If sickle cell disease is diagnosed in the fetus, about 50% of parents elect therapeutic abortion.

5. RESULTS

5.1 Effectiveness

5.1.1 Effectiveness of screening tests

a. MCV / Red cell indices.

In a study of 1 286 antenatal women, the sensitivity and specificity of MCV and MCH for determination of carrier status was 98% and 92% respectively. MCV and MCH together are suitable for further rapid confirmation of carrier status, since individually their sensitivity and specificity were lower (Maheshwari M, 1999). In another study, MCV and MCH were found to be useful as preliminary Thalassemia screening tests. However, in three of the six people provisionally diagnosed as having delta-beta-Thalassemia trait, an overlap of MCV and MCH values with the normal range occurred (Berdoukas V et al, 1983).

Another study in Thalassemia-traits individuals, showed that the simple tests (MCV, MCH, HbA2) or osmotic fragility were found not to be equally superior to other tests. The one tube osmotic fragility test has been found to be suitable for use in future Thalassemia screening programmes (Khin E H et al, 1992; Camagna A et al, 1974).

The measurement of volume and haemoglobin concentration of individual red blood cells can differentiate between iron deficiency anemia and thalassemia. Using the microcytic-hypochromic ratio to diagnose thalassemia found 94% predictive value with sensitivity of 94% and specificity of 92.3% (D'Onofrio G et al, 1992)

A two-step procedure for the detection of non-alpha thalassemia in mass screening programmes, involving red cell morphology and a one tube red cell osmotic fragility test to eliminate non-thalasssemic samples followed by determination red cell indices and haemoglobin studies, has a detection rate of 99.65 % (Silvestroni E & Bianco I, 1983)

b. Naked eye single tube red cell osmotic fragility test (NESTROFT)

NESTROFT has a sensitivity ranging from 91%-98.7%, specificity 66.6%-100%, positive predictive 55% - 87% and negative predictive values 96.5 - 99.6% (Thool A et al, 1998; Manglani M et al, 1997; Unpublished Malaysia survey, 1996; Thomas S et al, 1996; Raghavan K et al, 1991).

A study on screening for beta-Thalassemia trait in subjects from Greece, Yugoslavia and Thailand demonstrated the effectiveness of the one tube method of osmotic fragility using 0.32% saline as compared to 0.36% saline and tyrode (Kattamis C et al, 1981).

A local survey found that a combination of NESTROFT and red cell indices increases the sensitivity and negative predictive value to almost 100 % (Zuraidah, unpublished). NESTROFT with red cell indices like MCV < 70fl and RBC count >4.5x10(12)/l) has been found to be the most sensitive and specific test. NESTROFT combined with MCV<80fl proved 100% sensitive,

however the combination was not cost effective ((Manglani M et al, 1997). The effectiveness of the combination was supported by a comparative analysis of NESTROFT with red cell indices, like MCV (<70fl) and RBC count (>4.5x10(12)/l), where it was concluded as the single most effective, inexpensive and easily reproducible test of population screening for beta-Thalassemia trait (Gomber S et al, 1997). However, a survey found that of the 16% who were positive by preliminary screening using NESTROFT and red cell indices, only 4.5% were confirmed as carriers by HbA2 estimation.

c. Hemoglobin A2 Estimation

A bio variant Hemoglobin testing system was able to quantify hemoglobin A2 and F and flag other hemoglobin variants with a mean carry-over of less than 0.25%, for hemoglobin F, S and A and less than 2.08% for hemoglobin A2. This instrument never failed to indicate the presence of an abnormal hemoglobin in 271 selected samples. (Waters M et al, 1998).

Assay of Hb A2 by means of DE-52 microchromatography in haemosylates from 285 normal subjects and 223 beta-Thalassemia heterozygotes found no overlap between both groups. Comparable results were observed analysing whole blood samples collected in capillary tubes from 550 normal subjects and 295 beta-Thalassemia heterozygotes. These results demonstrate that this technique is useful in a screening program for beta-Thalassemia traits (Galanello R., Cao A, 1977). A statistical analysis of Hb A2 and Hb F levels in 1340 normal subjects and 356 heterozygous subjects with beta-Thalassemia, found normal distribution of Hb A2 and a gaussologrithmic distribution of Hb F. More than 97% of those heterozygous for beta-Thalassemia have Hb A2 level outside the overlapping region, which permits diagnosis. Of the remainder, only the 'silent carriers' do not manifest the usual hematological characteristics of beta-Thalassemia minor. Determination of globulin chain synthesis has to be carried out to diagnose this group, and this test is reserved for subjects considered 'high risk' (Beris P et al, 1980)

d. Hb electrophoresis using HPLC or isoelectric focusing

A study found that the introduction of universal testing by HPLC into British laboratories could be cost-neutral and has potential benefits. Automation could be be used to release skilled staff for other tasks within the laboratory (Phalau L & Bain K, 1999). Another study found that HPLC has high sensitivity and specificity and has proven to be clinically accurate, eliminating the need for a second screening test, and accurately discriminating beta-Thalassemia conditions (Lorey F & Vichinsky E, 1990).

e. DNA mutation analysis

Amplification refractory mutation system (ARMS) was found to confirm diagnosis in 98.3%, and provide a complete diagnosis in 94.2%, and thus found to be an inexpensive, robust and non-isotopic method for prenatal diagnosis of beta-Thalassemia in India (Saxena R et al, 1998)

f. Polymerase chain reaction (PCR)

A study of DNA in 24 chorionic villi samples, determining the presence of alpha-globin genes by PCR, showed the optimal number of amplifications for accurate diagnosis to be 50 cycles. It was concluded that DNA amplification using PCR offers more rapid analysis than gene mapping and requiring only 1 microgram of DNA for analysis (Tan JA, 1991).

PCR can also be used in a multiethnic population as evidenced in a study for the detection of beta-Thalassemia mutation in the Singaporean population, which detected approximately 95% of the mutations (Savage D et al, 1995).

PCR has been found to be useful in the diagnosis of alpha-Thalassemia, being able to identify carrier status in 82% patients with microcytosis, not related to iron deficiency or Beta-Thalassemia (Sivera P et al, 1997).

PCR when compared with hemoglobin H (HbH) inclusion test with in routine screening for alpha-Thalassemia, was found to be more sensitive. Thus, in areas with high prevalence of (--SEA/) deletion like Hong Kong, it has been suggested that PCR replace. HbH inclusion test in screening for alpha Thalassemia (Chan Y et al, 1996).

g Other Screening modalities

The sensitivity of anti-zeta antibody test was found to be 95% with a specificity of (--SEA) deletion mutation of 100% in a study of screening (--SEA) double alpha-globin deletion in South East Asia and Southern China. Although, it did not detect total alpha-deletions, it identified those alpha-Thalassemia 1 carriers due to (--SEA) deletion (Luo H et al, 1993).

A study to assess the safety and efficacy of diagnostic cordocentesis at mid-pregnancy, found that 97% were done successfully at the first attempt, and of these 1281 cases, 184 fetuses had severe disease (Tongsong T et al, 2000).

5.1.2 Effectiveness of Screening Programmes

Screening can be justified as a public health measure because it meets several criteria defined by the National Research Council and other agencies. These criteria include public benefit and acceptance, the benefits of screening outweigh the costs, appropriate and available public education, satisfactory test methods, lab procedures, reporting procedures, counseling and evaluation. The priority of screening programs can be based on the incidence of the threatened condition and its severity, the availability of techniques for discovery, diagnosis, control, and perhaps prevention (Chadwick R et al,1998).

A Thalassemia screening program for pregnant women in Southern Thailand found that in a total of 5078 pregnant women there were 40 clinical cases, of which 33 (82.5%) were diagnosed prenatally, with genetic amniocentesis being the most acceptable method. 5 cases (12.5%) were misdiagnosed due to contamination of maternal blood cells in amniotic fluid, while questionable results were reported in 2 cases (5%). Thus, a screening programme was found to be feasible in the prevention and control of Thalassemia disease. (Kor-anantakul O et al, 1998). In the antenatal screening in pregnant women in Chiang Mai, Thailand, genetic counseling was offered to couples at risk. 181 carriers were identified from 7954 prengnancies, of whom 134 underwent cordocentesis. 33 were proven to have severe Thalassemia. This strategy identified all fetuses with severe Thalassemia with no false positive among screened couples and proven highly effective in the control of severe Thalassemia (Tongsong T et al, 2000). A similar study in Thailand described a prospective screening program of 12 680 women, where 459 couples at risk were identified, 302 pregnancies underwent cordocentesis and 53 (17.5%) fetuses were found to

have severe Thalassemia major (Wanapirak C et al, 1998). Another screening program in Thailand tests for carrier state by NESTROFT in pregnant women with no risk, with subsequent testing of the husbands, for those women with abnormal test results. A cordocentesis in the 252 pregnancies at risk found 60 fetuses with severe Thalassemia (Wanapirak C et al, 1998).

In Montreal, Canada the incidence of Thalassemia has fallen by 90-95% over the 20 years with the introduction of a screening programme. The rare cases born are mostly outside the target communities or to non-screened couples (Mitchell J et al, 1996).

A review showed that prospective carrier screening is ongoing in Cuba and Guadeloupe for sickle cell anemia in a very limited way, alpha-Thalassemia in some South East Asian populations and beta-Thalassemia in several Mediterranean at-risk populations. These programmes have resulted in increased knowledge on Thalassemia and its prevention by target population and marked decline of the incidence of Thalassemia major. Hematological methods followed by mutation detection by DNA analysis are used. Prenatal diagnosis is accomplished by mutation analysis on PCR-amplified DNA from chorionic villi (Cao A et al, 1998).

In Cyprus, Greece and Sardinia programmes based on heterozygote detection, counseling and feotal diagnosis have resulted in a decline of Thalassemia major births by 50-97%. The reasons for residual cases were mostly lack of information, and, less frequently, misdiagnoses or refusal of fetal diagnoses (Cao A, 1987). In the Sardinian population, first trimester diagnosis initially by fetal blood analysis followed by trophoblast or amniocyte DNA analysis, resulted in an increase in the acceptance rate. This program resulted in a decline in Thalassemia major births of 90% (Cao A et al, 1989).
In Hong Kong, where Thalassemia major is common, antenatal screening has been carried out since 1983. Yet new patients are still being diagnosed. A retrospective study of 34 severe beta-Thalassemia syndromes in two major public hospitals between 1990 and 1996 found that this was due to lack of maternal screening and paternal screening, late or no antenatal visit, and cross-border deliveries from Mainland China to Hong Kong (Lee C et al, 1998).

In a recent study, where 18 907 pregnant women were screened for abnormal hemoglobin and their partners tested, 77 pregnancies were identified as being at risk because the partner also was a carrier of abnormal hemoglobin. Prenatal diagnosis was offered to suitable candidates, being accepted by 25 couples (47%). Of 18 amniocenteses actually performed, 5 fetuses were found to have clinically significant hemoglobinopathies and one of these pregnancies was terminated. In another study comparing results of prenatal and neonatal haemoglobinopathy screening, of the 54 700 pregnant women screened, 1019 (1.9%) had a haemoglobinopathy trait, and 81 women with at-risk fetuses were identified. Of these, only 28 (35%) accepted amniocentesis for definitive fetal diagnosis. The 4 women who were carrying a fetus with Thalassemia major elected to terminate the pregnancy. However, there were a total of 21 cases of haemoglobinopathy, but only 7 were diagnosed prenatally, while 14 were discovered during the neonatal period. Thus, it was concluded that a combined prenatal and neonatal program would offer the maximum benefit to patients by adding prenatal counseling, parental options, education, and early complete diagnosis to neonatal screening (Schoen J et al 1993)

In European communities with a high prevalence of b-Thalassemia, it has been said that the birth rate of affected infants has declined significantly following the implementation of routine prenatal screening. A similar trend is seen in some North American communities that have introduced community education and testing for Thalassemia. However, time series studies do not prove that such trends are due specifically to the effects of prenatal screening.

5.2 Safety

It has been said that amniocentesis and cordocentesis have a small risk about 0.5 – 1.5% of bleeding, abortions and infections. A hospital screening program for Thalassemia, in Southern Thailand found that abortion occurred in one case (0.7%) (Kor-anantakul O et al, 1998). A study to assess the safety and efficacy of diagnostic cordocentesis in 1 320 women at 16-24 weeks of pregnancy, showed that procedure-related complications included transient bleeding at puncture site, transient fetal bradycardia, and rarely, chrioamnionitis and cord hematoma. The total fetal loss rate was 3.2% with procedure -related loss being 1%. The maternal blood contamination rates were higher when cord insertion was targeted (Tongsong T et al, 2000). With increasing experience, it has been said that fetoscope and placental blood sampling carries a risk of less than 1% fetal loss (Loukopoulus D, 1983)

5.3. Cost Implications of Screening.

Screening has been claimed to increase overall health-care costs, but the savings associated with not having to provide services and support for children with disabilities have not been considered (BC Office of HTA).

A costing study in Inner London found that antenatal screening with follow-up counseling could be self-financing at most levels of prevalence of haemoglobinopathy traits. There would be greater savings if a high proportion of traits were beta-Thalassemia. Antenatal screening would be considered cost-effective even at quite low levels of trait prevalence, since there are other benefits (Cronin E et al, 2000).

A local study on transfusion dependent thalassemia patients found that that the costs of repeated hospital admissions; blood transfusions, antibiotic therapy and expensive investigations, regular anti-chelating deferrosamine therapy; prolonged hormonal replacement therapy; treatment for complications such as insulin-dependent diabetes mellitus, cardiac failure, hepatitis and possibly HIV infections impose a substantial burden on the limited resources (George E, 1996).

Analysing the economic costs of a screening program, the cost per case prevented is approximately $6 700, which is slightly less than the annual cost of treatment of a patient. About 4% of the cost of treatment is incurred in the first 25 years of life for an affected individual (Scriver C et al, 1984; Moatti J et al, 1998). In Quebec too, it was found that the total direct cost per case prevented in the program is less than the cost for a single year of treatment for an individual with the disease. Sensitivity analysis accommodating demographic assumptions, participation rate, and discounting rates indicates that, even at rates of marriage, endogamy, and participation lower than observed in the current program, treatment costs will still exceed

prevention costs when discounting is set at conventional rates of 4% and 8%. (Ostrowsky J. & Scriver C, 1985).

A study in India showed that there is a heavy burden on families with children afflicted with Thalassemia is because, apart from caring for the chronically sick child, the costs of treatment may amount to 20-30% of their income (Sangani B et al, 1990) In Sri Lanka, management of Thalassemia required 5% of total health budget (de Silva 2000)

A review of an antenatal and neonatal screening programmes in Inner London found that the cost per test using isoelectric focusing and high power liquid chromotography are similar. At a rate of 16 traits per 1000 and 0.5 sickle cell disease (SCD) per 1000, there was no significant cost difference between universal and targeted programmes. However, below this prevalence, a targeted program would be cheaper, but is likely to miss cases of SCD. A greater use of prenatal diagnosis resulting in termination, and consequently fewer affected births, reduces the cost effectiveness of a universal screening programme. It has been suggested that screening services should aim to cover a generated annual workload of more than 25 000 births, preferably over 40 000 births annually (Cronin E et al, 1998).

A policy of no screening was compared to universal or targeted screening, with selective follow-up of homozygous or compound heterozygous infants, and complete follow-up of infants with clinically insignificant traits in Alaska. Among the selective follow-up options, it was found that targeted screening is the most cost-effective strategy. Universal screening is more cost-effective than targeted screening for several scenarios like high prevalence, a low screening test cost, and a high cost per screen associated with racial targeting. Among the complete follow-up options, both targeted and universal screening have similar costs (Gessner B et al , 1996).

A study comparing the cost of universal testing for variant Hbs and Beta-Thalassemia trait using HPLC, and selective testing using MCH as a screening test and HPLC as a diagnostic test found both costs to be comparable, as the higher reagent and instrument costs of HPLC were off-set by the lower labour costs (Plelan et al, 1999). A comparison of universal haemoglobin electrophoresis and selective use of haemoglobin, found that while the latter involves lower costs, it may not identify every carrier of a haemoglobinopathy trait. However, it may be appropriate in some populations.

If screening is carried out only for beta-Thalassemia, cost effectiveness ratios can be improved by using a blood count. Further improvement of cost-effectiveness ratios is obtained by limiting screening to ethnic groups most ‘at risk’, but such strategies raise ethical and acceptability issues (Moatti J et al, 1988).

A costing study in Israel considered the lifetime health care costs of persons born with Thalassemia which included home infusion services, chelating agents, hospitalization, operations, outpatient visits, laboratory tests, therapists and others as well as lost earnings and premature mortality costs. The benefit-cost ratio of the program to the health service is 4.22:1. This ratio increases to 6:1 when a societal perspective is adopted (Ginsberg G. et al, 1998). Another costing model found that within sensitivity analysis, the undiscounted lifetime cost of treating a beta-Thalassemia major patient ranged from approximately £188 000 to £226 000

(Karnon J. et al, 1999), while another study estimated it to be USD 284 154 (Ginsberg G et al, 1998).

5.4. Social, Ethical and Legal Implications.

5.4.1 Social implications

Prenatal screening has been described as tools, which provide 'choice', and thus, some degree of control over pregnancy and reproduction. The initial choice of prenatal screening would be as to whether or not to have the test, and subsequently whether to continue a pregnancy in which an anomaly is detected. The central goal of prenatal counseling is 'informed choice' (BC Office of HTA).

A study in India of 200 families with Thalassemia found that ignorance and prejudice in the community led to social isolation for families (Sangani B et al, 1990). It has been shown that after confirmation and proper counseling, 88.7% of women with affected fetuses opted for termination of their pregnancy, while the rest declined principally on religious grounds (Med S et al, 2000). However, it has been pointed out that, it is unlikely that increasing women's knowledge alone would solve the problem of anxiety that women experience when receiving positive screening results (BC Office of Health Technology Assessment 1998).

Looking at the long term effects of prenatal diagnosis on couples at high genetic risk of Thalssemia, a study in 102 couples found that only 30 % had a favourable outcome, with a significant number having difficulty in obtaining even a single healthy child. It is suggested that more research is needed to enable those involved to have better control of their reproductive outcomes (Petrou M. & Ward R, 2000).

There is also a lack of awareness of the importance of screening especially in developing countries. Most families had poor education, low socio-economic status and more than half of couples not aware of their genetic risk. There is a need to have a control program adapted to particular populations, with proper information and counseling, and appropriate financial resources to make it a success (Zahed L et al, 1997). In a study, in Pakistan, a year after the introduction of a screening service, although 72% were aware of the availability of a screening test, only 56% went go for a second prenatal diagnosis. The reasons provided were cost of test, fear of undergoing test and lack of a clear explanation (Ahmad S et al, 2000). It has been said that although screening does not provide a diagnosis, it is important to discuss with the couples involved the implications of using this screening test. Screening can also create unnecessary anxiety in a low-risk population. Positive test results may result in negative attitudes towards both their pregnancy, and towards the expected child. False-positives may result in damaged to emotional health throughout the pregnancy. Even with a negative result, there may be difficulties in reestablishing the positive emotional feelings during the pregnancy (BC Office of Health Technology Assessment 1998).

Physicians have to be sufficiently aware of the test parameters and diagnostic testing options, and to provide accurate and timely information to patients, directing them neither towards nor away from these testing options. Genetic counseling involves advising patients on the

difficulties that may arise from genetic disease. Patients must be informed of the medical facts, severity and prognosis of the genetic disorder, the risk of its reoccurrence, and the options available for the management of the disorder (BC Office of Health Technology Assessment, 1998).

It is the moral dilemma about abortion that often causes controversy in prenatal diagnosis. Prenatal diagnosis may be considered controversial because the results may be used to justify abortion, which is a controversial issue in today's society. Those who oppose it consider it to be contrary to the goal of medicine, which is to save lives. Those who support selective abortion do so because they wish to prevent suffering and disease (Chadwick R et al. 1998). It has been suggested that physicians opposed to abortion on moral grounds and therefore under difficulties in counseling their patients about screening, have an ethical obligation to refer their pregnant patients to a colleague (BC Office of HTA, 1998).

The role of religion pertaining to the termination of pregnancy needs to be considered. In Pakistan Islamic scholars have ruled that a pregnancy can be terminated before 120 days as gestation (Ahmad S et al, 2000). In Iran, with strong religious restrictions on abortion, and a high prevalence of Thalassemia major, alternative strategies have been considered. Screened was carried out with CBC and HbA2 level measurement. Where couples were both trait positive, 90% decided not to marry. No new cases of Thalassemia were detected in the children of screened population. Genetic counseling centre can help to prevent most Thalassemia cases (Ghanei M et al, 1997).

5.4.2 Ethical implications.

The debate over mandatory genetic screening is based on the disagreement over both the necessity and right of society to intervene in affected individuals' rights to procreate. So, the major question is whether genetic screening should be considered under the domain of public health policy, or, on the other hand, remain a private responsibility. It is generally agreed that there needs to be a trade-off between the rights of individuals and the rights of society. Since society's concern for the genetic welfare of the population based on the danger of genetic deterioration is yet unfounded, cost/benefit analyses are often employed as strictly utilitarian criteria to determine trade-offs point (Chadwick R et al, 1998).

It has been argued that this type of screening can be supported by the doctrine that the state can act to protect those that cannot protect them.

However, others argue that this screening is for the purpose of identifying ‘defective’ fetuses, and once identified, to abort them. It is suggested that instead of putting all resources into screening, it should also be supporting children with handicaps (BC Office of HTA).

There are human risks involved in all genetic screening programs like include labeling, discrimination, loss of self-esteem, prevention or damage to parent-child bonding, stigmatization, unnecessary anxieties and invasion of privacy. The impact on the individual and the family, the usefulness and cost of obtaining the information, and the availability of facilities and personnel need to be considered. Screening programs for sickle-cell disease have shown negative outcomes without proper planning and attention to detail (Chadwick R et al, 1998.).

5.4.4. Legal implications

Proper safeguards are necessary to protect or alleviate the human risks involved. Legislators, health officials, and society representatives must determine appropriate guidelines to ensure health measures. Legal liability issues have arisen with the advances in genetic screening. The issue of malpractice litigation reflects the increased expectations of high-quality, disappointment-free medical care. It has been pointed out that the legal principles not only require a well-planned screening program, but also, all the stipulations of informed consent in the doctor-patient relationship must be met. In order to develop an informed decision-making client, counseling for genetic disorders becomes necessary (Chadwick R et al, 1998).

Legal action may be brought about for wrongful birth brought by parents with abnormalities against the physician or midwife who allegedly failed to provide appropriate prenatal counseling or information. There may be claims that no adequate warning of the potential problem to their child was given, and the lack of timely information prevented them from obtaining an abortion. Consequentially, there would be claims for damages for the parents themselves as well as for support and care of their child (BC Office of Health Technology Assessment 1998).

6 CONCLUSION

There is sufficient evidence to support the screening for Thalassemia, but there are major ethical and religious issues among the Muslim population

7. RECOMMENDATIONS

Due to the ethical and religious issues surrounding prenatal screening, invasive diagnostic procedures and termination of pregnancies, routine antenatal maternal serum screening for Thalassemia is not recommended. However, screening should be made available to women who request for the test.

MATERNAL SCREENING FOR FOETAL ABNORMALITY – OVERALL RECOMMENDATIONS

Due to the ethical and religious issues surrounding prenatal screening, invasive diagnostic procedures and termination of pregnancies, a national programme of routine antenatal maternal serum screening for Down's syndrome, neural tube defects and Thalassemia is not recommended. However, screening should be made available to women who request for the test.

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EVIDENCE TABLE : SCREENING FOR BETA-THALASSEMIA TRAITS – TECHNICAL FEATURES

No	Author, Title, Journal Year,	Study design, Sample size, follow-up.	Outcome & characteristics.	Grade & Comments
1.	Old J. Haemoglobinopathies. Prenat Diagn 1996 Dec; 16(13):1181-6.	Commentary	The main requirements for methodologies providing molecular diagnosis are speed, cost, convenience and the ability to test for multiple mutations simultaneously. For beta-Thallasemia mutations the procedures that meet these requirements are the amplification refractory mutation system and the reverse dot-blot hybridization system. For alpha-Thallasemia the technique of gap PCR is useful for targeting specific deletion mutations but the Southern blotting remains the standard diagnostic test.	
2.	Galanello R. Sollaino C et all. Alpha-thal. carrier identification by DNA analysis in the screening of thalassaemia. American journal of haematology 1998, Dec; 59(4):273-8..	526 adult.	Alpha-thal carrier identification by PCR based method in patients with: 1. reduced MCV 2. reduced MCH 3. normal HbA2 and HbF 4. normal se.Iron. Conclusion : PCR is fast and accurate to identify alpha-thal carrier.	
3.	Kleman K. Lubin B. et al./ Experience with newborn screening using isoelectric focusing. Pediatrics 1989 May;83(5pt2);852	Review	Electrophoretic techniques are used for hemoglobinopathy diagnosis. Confirmation of the hemoglobin variants is necessary. Currently, citrate agar electrophoresis is the most available, but high performance liquid chromatography is highly recommended in a reference laboratory. Thin layer iso-electric focusing is an excellent technique that can be easily adapted to large-scale newborn hemoglobinopathy screening. Although the initial instrument cost can be twice as much as the cost for standard electrophoretic equipment, cost-effectiveness for newborn screening is considerable because repeat analysis for uninterpretable results is not necessary. Resolution of hemoglobin is much better with thin layer isoelectric focusing than for any of the other electrophoretic	

No	Author, Title, Journal Year,	Study design, Sample size, follow-up.	Outcome & characteristics.	Grade & Comments
			methods currently available, and thin layer isoelectric focusing is the best method to use to establish a definitive diagnosis using newborn blood samples. Cord blood samples may be contaminated with maternal blood, and evaluation of Hb A2 levels in such samples can serve as the method to detect contamination. Follow-up testing is required regardless of the method of blood collection. .	
4.	Mitchell J., Scriver C et al. 20 years outcome analysis of genetic screening programs for beta-thalassemia disease carriers in high schools. Am J Hun Genet 1996 Oct;59(4):793-8.	Prospective study. 25 274 students.	women with no risk and testing the husbands of the women with abnormal tests. A pregnancy in which both of the couple were carriers was considered at risk. Cordocentesis was performed in 554 pregnancy at risk. 252 from retrospective screening and 302 from prospective screening respectively, 60 of 252 of the first group had severe thallasemia. In the prospective screening program of 12 680 women, 459 risk couples were identified, 302 pregnancies underwent cordocentesis and 53 (17.5%) had severe thalassemia. This strategy enable us to identify 113 cases of severe Thallasemia (Hb Bart 60, Beta-Thallasemia 53) from 554 cases at risk. In conclusion, the strategy proves valuable in the control of severe thalassemia. This extensive experience suggests the strategy be considered an effective way in the control of severe thalassemia in high prevalence area. -In Montreal programs for education, screening and counselling of senior-high-school students, in populations at high risk for beta-thalassemia have existed for > 20 years. 25 274 students were screened for beta-thalassemia, 693 carriers (frequency 1:36). All carriers identifed in the high-school screening programme remembered their status, had their partner tested if they did not already known ther were carrier couple, and took up the options for reproductive counselling/prenatal diagnosis. In Montreal, the current origin of all couples using prenatal diagnosis for beta-thallasemia disease is the corresponding genetic screening program, whereas, at the beginning of the programs, it was always	

No	Author, Title, Journal Year,	Study design, Sample size, follow-up.	Outcome & characteristics.	Grade & Comments
			because there was a history of an affected person in the family. Incidence of the disease has fallen by 90-95% over the 20 years. The rare cases are born (with 2 exceptions) outside the target communities or to non-screened couples.	
OTHER SCREENING MODALITIES				
5	Christian Medical College Hospital, Tamil nadu, India. Prenatal diagnosis of beta-thalassemia mutation using the reverse dot blot technique. Natl Med J India 1996 May-Jun;9(3):150. 47	Case report. 1 12 week old fetus, delivered later as normal.	Beta-thalassemia is the most common genetic disorder and a number of mutations causing this disease have been reported. Since affective treatment of thalassemia major is complicated and very expensive, prenatal diagnosis has become an important option for those at risk of having an effected feotus. Report the use of a rapid hybridization method called 'reverse dot blot' for detection of specific mutations of the beta-globin gene. DNA was obtained from a 12 week old fetus by chorionic villus sampling and was amplified using specific primers by the polymerase chain reaction and analysed by the reverse dot blot test. Results were available within 36 hours after sampling. The father and mother were found to be heterozygous for codon 15 (G-A) mutation of beta-globin gene. The fetus was normal. In conclusion the reverse dot blot is a rapid and reliable technique for mutation detection in the beta-globin gene and can be useful for antenatal diagnosis.	
6	Melis A. Cao A. et al. Hematological Charactyeristics of sardinian alpha-thalassemia carriers detected in a population study. Acta Haematol 1980; 6391:32-6	Randomise case-control study. 88 subjects.	88 adults with Thalassemia-like indices, normal serum iron and normal hemoglobin (Hb) A2 and F levels, diagnosed in a mass screening had Hb H inclusion bodies studies (65 subjects) or Hb H inclusion bodies studies and globin chain synthesis analysis (23 subjects). Hb H inclusion bodies were found to be a reliable test for alpha-thal trait identification, resulting in positive inapproximately 70% of suspected carriers. The alpha-thal carrier defined by Hb H preparation or by globin chain synthesis had significant reduction in the mean Hb level, hematocrit, mean corpuscular hemoglobin and a significant increase in mean red cell count, but there are some overlap with controls .	

No	Author, Title, Journal Year,	Study design, Sample size, follow-up.	Outcome & characteristics.	Grade & Comments
7	Luo H.Y, Chui D.H et al. A novel monoclonalantibogy based test for alpha-thalassemia-1 carriers due to the (--SEA/) deletion. Blood 1988 Nov; 72(5):1589-94.	Case controlled trait 30 sample each	The presence of minute amounts of embryonic zeta-globin chains in adult hemosylates is a marker for carriers of alpha-thalassemia 1 resulting from (--SEA/) deletion. A murine monoclonal antihuman embryonic zeta-globin chain antibody, 8E8. By using this antibody, we have now established a slot-blot immunobinding assay fir rapid detection of zeta-globin chains in adult hemosylates. Zeta-globin chains were found to be present in 30 blood samples obtained from individuals who were carriers of alpha-thalassemia-1. In another 30 blood samples from individuals who were not carriers of the (--SEA/) deletion, zeta-globin chains were not detected. This simple diagnostic test can be used in appropriate populations to identify those couples at risk of conceiving fetuses afflicted with Hb Bart's hydrops fetalis syndrome due to homozygous alpha-thalassemia.	
8	Chambers K, Chapman C et al. Use of a low pressure liquid chromatography system for haemoglobinopathy screening. Clin Lab Haematol 1993;15(2):119-28.	Randomised control study, 252 samples.	A closed low pressure liquid chromatography system (LPLC) suitable for haemoglobinopathy screening. The biochemical principles applied to the separation of haemoglobin A2 (HbA2) and haemoglobin variants. The instrument offers three modes of use including a fast haemoglobin elution, a variant screen and a HbA2 assay for thalassemia screening. The fast screen isolates all of the common haemoglobin variants except HbE which elutes with HbA. This mode is a more rapid alternative to the Sickledex test. The variant screen produces a wider separation of abnormal variants giving an identification and quatitation for each. The HbA2 assay separates this minor fraction from all other haemoglobins giving an accurate percentage. Abnormal variants are also separated. To validate the HbA2 assay 252 samples were assayed by the cellulose acetate electrophoresis/elution method and LPLC with a correlation of).932. The system provides an accurate and sensitive alternative to traditional manual chromatography and electrophoresis methods. The automated sampler allows	

No	Author, Title, Journal Year,	Study design, Sample size, follow-up.	Outcome & characteristics.	Grade & Comments
			batches from 1-99 samples to be processed with significant savings in operator times.	
9	Kuliev A. Verlinsky Y. et al. Birth of healthy children after preimplantation diagnosis of thalassemia. J Assist reprod Genet 1999 Apr;16(4):207-11.	Prospective study. 166 oocytes.	Preimplantation genetic diagnosis (PGD) allows couples at risk of having children with thalassemia to ensure the healthy outcome of their pregnancy. 17 PGD clinical cycles were initiated for Cypriot couples at risk of having children with different thalassemia mutations. Unaffected embryos for transfer were selected by testing oocytes, using first and second polar body (PB) removal and nested polymerase chain reaction analysis followed by restriction digestion. RESULTS: Unaffected embryos were selected in 16 of 17 PGD cycles. Of 166 oocytes studied from these cycles, 110 were analysed by sequential analysis of both the first and the second PB, resulting in preselection and transfer of 45 unaffected embryos. This resulted in 7 pregnancies and in the birth of 5 healthy thalassemia-free children. The embryos predicted to have the affected allele were not transferred. Analysis of these embryos confirmed the PB fiagnosis. CONCLUSIONS: Sequential first and second PB testing of oocytes is reliable for PGD of thalassemia and is a feasible alternative to prenatal diagnosis in high-risk populations.	
10	Harada F, Chui D et al. Anti-zeta antibody screening for alpha – thalassemia using dried filter paper blood. Biochem Med Metab Biol 1994 Feb;5(1):80-4. 47	Randomised control study. 91 microcytic samples.	The most common alpha-thalassemia is the (--SEA) double alpha-globin deletion. The (--SEA) deletion spares the embryonic zeta-globin genes and causes traces of zeta-peptide to persist throughout life. A colorimetric monoclonal anti-zeta antibody test for raised zeta-peptide has detected the (--SEA) deletion, but not deletions of the entire alpha-globin region with loss of the zeta-globin genes. Elutes from dried blood spots had the same anti-zeta antibody colour reaction as whole blood, even after storage at 4 degrees C for up to 77 days. The anti-zeta antibody test was positive in 24 of 91 microcytic samples (mean corpuscular haemoglobin < 24pg), including four with iron deficiency, it was negative in 26 provisionally	

No	Author, Title, Journal Year,	Study design, Sample size, follow-up.	Outcome & characteristics.	Grade & Comments
			diagnosed alpha-thalassemia heterozygotes and all 32 non-microcytic samples. Southern blot analysis and a specific SEA-polymerase chain reaction test confirmed that 18 anti-zeta antibody positive samples and 1 anti-zeta antibody negative sample had the (--SEA) deletion. Two anti-zeta antibody negative microcytic samples had the (--Fil) total alpha-globin region deletion, 2 had single alpha-gene deletions, 22 others may also have total alpha-region deletion. The anti-zeta antibody test can detected the (--SEA) deletion in dried blood samples, even after prolonged storage, and specificity was very high and sensitivity was 95%. This simple inexpensive test can conveniently screen samples collected at a distance from a central laboratory.	
11	Skogerboe K, Tait J et al. Screening for alpha-thalassemia. Correlation of haemoglobin H inclusion bodies with DNA-determined genotype. Arch Pathol Lab Med 1992 Oct;116(10):1012-8.	Prospective study. 80 patients.	The most routine screening for alpha-thalassemia can be performed with three simple tests; 1. The brilliant cresyl blue inclusion test – reliably detects couple at risk for haemoglobin Bart's hydrops fetalis, measurement of number of inclusion bodies. 2. Erythrocyte indices, and 3. iron studies – found evidence of elevated serum ferritin levels in many patients with deletion of two or three alpha-globin genes. Analysis with DNA probes is needed in only some circumstances.	
12	Rady S. Romeo G. et al. Identification of Mediterranean beta-thalassemia mutations by reverse dot-blot in Italian and Egyptians.	Prospective study. 246 subjects.	Beta-thalassemia is a significant public health problem in Egypt where over 1000 of the annual 1.5 million newborns are expected to be affected with this disorder. A preventive program should be multi-faceted with its technique component based on carrier screening and prenatal diagnosis through mutation detection. In addition, it should have an information and educational component with the aim of	

No	Author, Title, Journal Year,	Study design, Sample size, follow-up.	Outcome & characteristics.	Grade & Comments
	Hemoglobin 1997 Jan; 21(1):59-69.		increasing public awareness of the disease. Proper selection of the techniques to be utilised in such a program is highli important. The appropriate technique to be used in screening should be reliable, simple and cost-effective. It should also circumvent the problem of marked heterogenecity of the disease in Egypt. The reverse dot-blot technique has been used in the present study for the characterization of mutations in 138 Italian and 108 Egyptian thalassemia chromosomes, confirming its reliability as a screening method. The technique is now in routine use for thalassemiaia diagnosis in the Microcitemia Centre of Galliera Hospital in Genoa, Italy. Based on this result, we recommended the reverse dot-blot method as the technique of choice in the preventive program of this disease in Egypt.	

EVIDENCE TABLE : SCREENING FOR BETA-THALASSEMIA TRAITS – EFFECTIVENESS

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
EFFECTIVENESS OF SCREENING TEST				
1	Tan J.A. et al An evaluation of the polymerase chain reaction for detection of alpha-globin genes in the prenatal diagnosis of alpha-zero thalassemia. Ann Acad Med Singapore 1991 Mar;20(2):251-4.	Randomised controlled trial. 24 chorionic villi samples.	Homozygous alpha zero-thalassemia results in the fatal disease Bart's hydrop fetalis. 3-4% carry the alpha-thalassenia genes, prenatal diagnosis of thalassemia is essential. Polymerase chain reaction (PCR) enables selective amplification of the 136 base pair region within the alpha-globin gene cluster, as a routine test for prenatal diagnosis of homozygous alpha zero-thalassemia.Confirmation of PCR results was performed using DNA gene mapping and electrophoresis of cord blood. DNA was extracted from 24 chrionic villi samples and the presence of alpha-globin genes was determined by PCR. Result showed that the optimal number of amplifications for accurate diagnosis was 50 cycles. Using PCR at less than 50 cycles of amplification (eg.35 cycles), false positive results were obtained in 30% of	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
			cases. Concluded that DNA amplification using the PCR offers an accurate method of prenatal diagnosis of alpha zero-thalassemia. Its advantage over the more establish gene mapping method include a more rapid analysis (3 days compared with 10 days by gene mapping) and the requirement of only minute amounts of DNA (1 microgram) for analysis. It is essential that the optimal number of amplification cycles be established so that false positive results may be avoided.	
2	Waters M. Richards J. et al. An evaluation of the Bio-Rad Variant Haemoglobin Testing System for the detection of haemoglobinopathies. Clin Lab Haematol 1998 Feb;20(1):31-40.		The bio-Rad variant Haemoglobin Testing system is an automated analyser which uses the principle of cation exchange high performance liquid chromatography. This evaluation was undertaken to examine the effectiveness of the instrument as a screening mechanism to assist in the diagnosis of haemoglobinopathy. The ability to quantify haemoglobin A2 and F and to flag other haemoglobin variants were tested. Within batch precision was excellent and between batch precision was good. Linearity and sensitivity compared favourably with the manufacturer's published rabges. The mean carry-over for haemoglobin F, S and A was less than 0.25%. The mean carry over for haemoglobin A2 was 2.08%. this higher figure reflected the smaller absolute difference between the high and low samples for this parameter. The instrument never failed to indicate the presence of an abnormal haemoglobin in 271 selected samples. The instrument was reliable throughout the evaluation and at no time was a run aborted.	
3	Manglani M, Mhaskar V et al. 'NESTROFT" – an effective screening test for beta thalassemia trait.	Prospective study. 2 525 subjects.	NESTROFT (sensitivity 94.4%) as a single screening parameter was superior to any other evaluated parameters individually (red cell indices MCV<75fl sensitivity 87.3%, Mentzre's fraction sensitivity 66.2% and discriminant functions (DF1-4) sensitivity 47.2-55.6%). NESTROFT has emerged as the single most effective, inexpensive and easily	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
	Indian Pediatr 1997 Aug;34(8):702-7.		reproducible test of population screening for beta-thallasemia trait. NESTROFT with red cell indices (ie. MCV < 70fl and RBC count >4.5x10(12)/l) revealed to be the most sensitive and specific test. NESTROFT in combination with MCV<80fl proved 100% sensitivity, however the combination was not cost effective. Subjects who are NESTROFT 'positive' or 'doubtful' deserve further investigation. NESTROFT is a sensitive, easy to perform, simple, inexpensive, does not required sophisticated equipment, cost effective, rapid and reliable screening test for detection of Beta-Thallasemia trait in a population. -.	
4	Gomber S, Madan N et al. Validity of NESTRIFT ib screening and diagnosis of beta-thalassemia trait. J Trop Pediatr 1997 Dec;43(6):363-6.	Randomised controlled trial. 253 childrens.	Validity of NESTROFT was evaluated on 253 individuals divided into 2 groups. Group 1 93 individuals belonging to family in which at least one of the children suffering beta-thalassemia major and group2 consist of 160 normal children. The sensitivity of NESTROFT was 95.6% (group1) and 85.7% (group2), specificity was 84.2% (grp1) and 81.7% (grp2), with negative predictive value as high as 99.2% in general population. Comparative analysis of NESTROFT with red cell indices, ie. MCV (<70fl) and RBC count (>4.5x10(12)/l) revealed it to be the most sensitive and specific test. NESTROFT has emerged as the single most effective, inexpensive and easily reproducible test of population screening for beta-thalassemia trait	
5	Thomas S, Chandy M et al. NESTROFT as a screening test for the detection of thalasemia and common haemoglobinopathy – an evaluation against a high	Randomised control study. 137 patients	The NESTROFT was evaluated against a HPLC method for its usefulness in screening for beta-thalassemia and some common haemoglobinopathies. Blood samples from 137 patients with suspected haemoglobinopathies were analysed by both methods. Among 63 patients with heterozygous beta-thalassemia on HPLC, NESTROFT was positive for 49, 'doubtful' for 13 and negative for 1. Of the 32 Paatients with other haemoglobinopathy , 28 were positive on NESTROFT and 4 were 'doubtful'. Of the 42 'normal' samples,	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
	performance liquid chromatography method. Indian J Med Res 1996 Aug;104:194-7.		NESTROFT was positive for 6, 'doubtful' for 8 and negative for 28. This test showed an overall sensitivity of 98.7%, specificity of 66.6%, positive predictive value of 87% and negative predictive value of 96.5%. In conclusion, NESTROFT is a suitable test for screening for beta-thalassemia and the common haemoglobinopathy. It is easy to perform, simple, inexpensive and does not require sophisticated equipment. Subject who are NESTROFT 'positive' or 'doubtful' deserves further investigation. The Naked Eye Single Tube Red Cell Osmotic Fragility Test (NESTROFT) was successful in detecting 105/110 subjects with beta-thalassemia traits. The sensitivity of the test was 95.5% and specificity was 87%. The predicitive value of the positive test was 70.5% and that of the negative test was 98.3%. The test proved to be simple, cheap, easy to perform and adaptable for field surveys, coming close to an ideal screening test for beta-thalassemia minor.	
6	Kattamis C. Pootrakul S et al. Effectiveness of one tube osmotic fragility screening in detecting beta-thalassemia trait. J Med Genet 1981 Aug;18(4):266-70.	Prospective study. 1371 subjects.	The effectiveness of the one tube method of osmotic fragility with three buffered solutions (0.32% saline,).36% saline, and tyrode0 as a screening for beta-thalassemia trait was evaluated in several groups of subjects from Greece, Yugoslavia and Thailand. The results clearly demonstrated that 0. 36% saline is the most sensitive and effective solution since it can detect 96 to 100% of heterozygous with beta-thalassemia, compared to about 80% with both 0.32% saline and tyrode. However. 0.36% saline gave false positive results in normal subjects and was also positive in haematological disorders, which influence osmotic fragility. The screening test with 0.36% saline was applied more precisely in 1371 subjects. The test was false positive in 41 (9.1%) of 455 normal subjects while of 438 confirmed heterozygotes with beta-thalassemia it was positive in 431 (98%) and negative in only 7 (2%). The test	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
			was also found to be positive in 80% of patients with iron deficiency anemia and alpha-thalassemia trait, in 68% of patients with Hb E trait, in 40% of patients with Hb S traits, and in 78% of heterozygous with rare haemoglobin variants. The increased sensitivity and effectiveness of 0.36% saline in detecting beta-thalassemia trait and otjer disorders influencing osmotic fragility as compared to 0.32% saline and tyrode solutions was also confirmed in a study of 384 unselected schoolchildren.	
7	Luo H, Hsia Y et al. Detection of the (--SEA) double alpha-globin gene deletion by a simple immunologic assay for embryonic zeta-globin chains. Am J Hematol 1993 Sep; 44(1):22-8.	Cross-sectional study. 225 peripheral blood samples.	The sensitivity of anti-zeta antibody test was 95%; and specificity for the (--SEA) deletion mutation was 100%. This test provides rapid, simple, and reliable screening for (--SEA) double alpha-globin deletion, although it does not detect the (--Tot) total alpha-deletions. This simple test can identify individuals who are alpha-thalassemia 1 carrier due to (--SEA) deletion. It is proposed that this test should be made available in South-East Asia and Southern China, in order to identify couples who are at risk of begetting fetuses afflicted with homozygous alpha-thalassemia.	
8	Berdoukas V. Raddatz B. et al. Community-initiated screening programme for beta-Thalassemia. Med J Aust 1983 Aug 6;2(3):129-31.	Prospective study. 795 cases screened.	The red cells of individuals with the alpha-thalassemia, beta-thalassemia, and haemoglobin lepore traits all had mean corpuscular volume (MCV) of less than 76 fl, and a mean corpuscular haemoglobin (MCH) of less than 25 pg, thus confirming the usefulness of these indices as a preliminary thalassemia screening test. However, in three of the six people provisionally diagnosed as having delta-beta-thalassemia trait, an overlap of MCV and MCH values with the normal range occurred.	
9	Camagna A. Zapponi G et al. A critical analysis of	Case-control study. 25 normal and 25 thalassemia cases	The significance of haematological test has been measured on statistical base. The purpose of such inquiry is the simplification of diagnosing thalassemia. A pilot research has been performed on a sample of 25 ‘normal’ and 25	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
	several haematilogical tests for diagnosis of thalassemia		'thalassemia' cases. The results show that some of the tests are particularly fit to diagnosis. There are reasons to consider best tests, in the order, HCH. HbA2, MCV, Hb, Recticulosite, Haematocrit, MCHC. By combining such tests one obviously obtains more reliable results. The 'doubtful' cases can be best treated by adding in a HbA2 test or G.R test. In the last case one obtain Hb/G.R and Haematocrit/G.R, that is MCH and MCV.	
10	D'Onofrio G, Mango G et al. Automated measurement of red blood cell microcytosis and hypochromia in iron deficiency and beta-thalassemia trait. Arch Pathol Lab Med 1992 Jan;116(1):84-9.	Randomised control study. 119 patients with iron deficiency anemia and 172 patients with beta-thalassemia traits.	Some routine red blood cell (RBC) and indexes (count, mean volume, volume dispersion and mean haemoglobin concentration (HGB) can be used to differentiate iron defeciency from heterozygous beta-thallasemia. A number of formulas that incorporates two or more of these measurments been described to amplify such differences. The H*1 haematology analyzer directly measures volume and HGB concentration of individual RBCs. The ratio between the percentage of microcytes and the percentage of hypochromic cells provided by the H*1 (microcytic-hypochromic ratio) was useful in differentiating the two types of microcytic anemia. Iron deficiency erythropoiesis is characxterised by production of RBCs with severely decrease HGB concentration, while microcytes of beta-thalassemia trait are generally smaller, with a more preserved HGB concentration. With a disriminant value of 0.9, the discriminant efficiency of the microcytic-hypochromic ratio was 92.4% (95% confidence interval, 88.8% to 95.2%), higher than that of the five previously described discriminat formulas and simple RBC measurments. A microcytic-hypochromic ratio lower than 0.9 demonstrate high sensitivity (94%), specificity (92.3%), and predictive value (94.0%).	
11	Khin E. H., Thien T.M. Thalassemia in the out-	Prospective study. 133 patients with Thalassemia	The mean values of haemoglobin concentration, packed cell volume (PCV), mean cell haemoglobin (MCH) and mean cell volume (MCV) were significantly lower than normal, but the	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
	patient department of Yangon Children's Hospital in Myanmar; basic hematological values of Thalassemia traits. Southeast Asian J trop Med Public Health 1992 Jun; 23(2):264-8.	traits.	MCHC was the same as normal. Increase osmotic resistance tested in0.36% buffered saline was detected in 81-97%. High levels of HbA2 (>3.5%) were found in 93%, whereas HbF was increase only in 23%. Although the mean red cell count (RBC) was significantly higher than normal, only 79% of thalassemia traits were detected if the RBC count of >5.0 x 10(12)was taken. Other discriminant functions such as MCH/RBC, MCV/RBC, (MCV)2 X MCH x 0.01 and MCV – (RBC/10(12)/l) – (5-Hb)- 3.4 or 8.4.All of them were found not to be superior to each of the simple tests (MCV,MCH<Hb2 or osmotic fragility. The one tube osmotic fragility test is a suitable test to be used in future thalassemia screening programs.	
12	Beris P. Miescher P et al. The detection of Thalassemia minor. Nouv Rev Fr Hematol 1980; 22(3):223-34	Randomised control study. 1340 + 356 subjects.	A statistical analysis was done on the level of Hb A2 and Hb F in 1340 normal subjects and 356 subjects with heterozygous for beta-thalassemia. Both had a normal distribution of Hb A2 and a gaussologrithmic distribution of Hb F. More than 97% of heterozygous for beta-thalassemia have a Hb A2 level outside the overlapping region, which permits diagnosis. Of the remaining 35, only the 'silent carriers' do not manifest the usual hematological characteristics of beta-thalassemia minor (hypochromia, microcytosis, diminished osmotic fragility, and erythrocytes with basophilic stipplings). To diagnose this group, it is necessary to determine globulin chain synthesis. This test is reserved for those subjects considered as 'high risk'.	
13	Savage D. Hui M. et al. Detection of beta-thalassemia mutations using DNA heteroduplex generator molecules.	Prospective study. Singaporean population.	A rapid polymerase chain reaction (PCR) based method for the detection of beta-thalassemia mutation was described. This method is based on visualization of unique DNA heteroduplex banding patterns, following non-denaturing polyacrylamide gel electrophoresis, resulting from hybridization between mutant PCR products and synthetic DNA heteroduplex generator molecules. Using Singaporean	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
	Br J Haematol 1995 Jul; 90(3):564-71.		population, which consists of Chinese, Malay and Asian Indian ethnic groups as a model, we have constructed and evaluated three DNA heteroduplex generator molecules for the detection of the common beta-thalassemia mutations found in the population. The results show that these three molecules are capeable of detecting approximately 95% of the mutations found in the Singaporean population. We propose that this technology may be applied as a alternative screening strategy fir beta-thalassemia mutations because it is tecnically simple, flexible, cost-effective, and requires only minimal laboratory resources.	
14	Chan Y,Chan C et al. Comparison of the HbH inclusion test and a PCR in routine screening for alpha thalassemia in Hong Kong. J Clin Pathol 1996 May; 49(5):411-3.	Randomised control study. 99 peripheral blood samples from Chinese patients with mean corpuscular volume < 80fl..	To compare the haemoglobin H inclusion test with a polymerase chain reaction (PCR) test in routine screening for alpha-thalassemia. Ninety nine peripheral blood samples from Chinese patients with mean corpuscular volume below 80fl were screened for alpha-thalassemia using the HbH inclusion test and by PCR utilising primers bridging the common deletion breakpoint of the South-East Asia (--SEA/) deletion. The HbH inclusion test was positive in 78 (79%) patients, 73 (93.7%) of whom carried tha (--SEA/) deletion on analysis of their DNA by PCR, as did one patient with a negative HbH inclusion test. These results suggest that in areas with high prevalence of the (--SEA/) deletion, such as Hong Kong, the HbH inclusion test can be replaced by PCR as the investigation of choice in screening for alpha-thalassemia.	
15	Lorey F, Vichinsky E et al. Universal screening for haemoglobinopathies using high erformance liquid chromatography: Clinical results of 2.2	Cross-sectional study. 2.2 million births screens.	In screening for haemoglobinopathies, HPLC achieves excellent sensitivity and specificity, while adding the very important quantitative element to the analysis. Due to the development of a rapid, automated HPLC system, screening 600 000 births per year in 1990, based on confirmatory testing for 97% of the initial positive results resulting from 2.2 million screens, HPLC has proven to be clinically accurate. HPLC provides a complete screening system, eliminating the	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
	million screens. Eur J Hum Genet 1994; 2(4):262-71.		need for a second screening test, and accurately discriminating beta-thalassemia conditions	
16	Thool A, Talib V et al. A simple screening test for the detection of heterozygous beta-thalassemia. Indian J Pathol Microbiol 1998 Oct; 41(4):423-6.	Prospective study, population screened.	NESTROFT (Naked eye single tube red cell Osmotic fragility test) with 0.36% buffered saline – Of the population screened, 42 cases were positive with NESTROFT, of which 40 were positive with confirmatory test for Beta-Thallasemia. Implies positive predictive value and specificity of 100%, negative predictive value of 83.3% and sensitivity of 95.2%.	
17	Maheshwari M et al Carrier screening and pre natal diagnosis of beta Thalassaemia Indian Pediatric 1996; 36: 1119-1125	1 286 antenatal women	the sensitivity and specificity of MCV and MCH for determination of carrier status was 98% and 92% respectively. MCV and MCH together are suitable for further rapid confirmation of carrier status, since individually their sensitivity and specificity were lower	
18	Silvestroni E & Bianco I A highly cost effective method of mass screening for thalassaemia Br Med J (Clin Res ED) 1983 Mar 26; 286 (6370):1007-9	289 763 student	Two step procedure for the detection ofnon alpha thalassaemias in mass screening programmes involve red cell morphology and a tube red cell osmotic fragility test, This esliminates the non thalassaemic sample following by determination of red cell indices and haemoglobin studies The detection rate is 99.65%	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
19	Raghavan K et al Evalution of naked eye single tube red cell osmotic fragility test in detecting beta thalassaemia trait Indian Pediatric 1991May; 28(5):469-72	Evalution	NESTROFT has sensitivity of 95.5% nd specificity 87%, positive predictive value of 70.5% and negative predictive value was 98.3%	
20	Galanello R. Cao A. et al. Quantitation of Hb A2 with DE-52 microchromatography in whole blood as screening test for beta-thalassemia heterozygotes. Acta Haematol 1977; 57(1):32-36.	Case-control study. 285 normal subjects and 223 beta-thalassemia heterozygotes.	Hb A2 was assayed by means of DE-52 microchromatography in haemosylates from 285 normal subjects and 223 beta-thalassemia heterozygotes. No overlap was found between both groups. Compareable results were observed analysing whole blood samples collected in capillary tubes from 550 normal subjects and 295 beta-thalassemia heterozygotes. Results demonstrate that this technique is useful in a screening program for beta-thalassemia traits.	
21	Phalau L., Bain K et al. An analysis of relative costs and potential benefits of different policies for antenatal screening for beta-thalassemia trait and variant haemoglobin. J Clin Pathol 1999 Sep; 52(9): 697-700.	Prospective study. 2000 antenatal patients.	1000 consecutive antenatal patient samples referred to two London teaching hospital laboratories for haemoglobinopathy testing were investigated using the standard procedures of the laboratory in question. When the standard procedures did not include high performance liquid chromatography (HPLC), this technique was added, in order to assess its diagnostic value and cost-effectiveness. A comparison was made between the costs and potential benefits of universal testing for variant haemoglobins and beta-thalassemia trait using HPLC and the costs and potential benefits of universal testing for variant haemoglobins and selective testing for beta-thalassemia trait using the mean haemoglobin (MCH) as a	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
			screening test and less automated techniques than HPLC for definitive diagnosis. RESULTS: The cost of the two policies were foung to be comparable, as the higher reagent/instrument costs of HPLC were off-set by the lower labour costs. Universal testing of 2000 consecutive samples did not disclose any extra cases of beta-thalassemia trait, which would not have been detected by universal screening and selective testing. However, six patients were found to have a haemoglobin A2 variant, which can interfere with the diagnosis of beta-thalasemia trait. CONCLUSIONS: The introduction of universal testing by HPLC into British laboratories could be cost-neutral and has potential benefits. If a higher cost is accepted then the greater degree of automation could be used to release skilled staff for other tasks within the laboratory.	
22	Saxena E, Jain PK, Thomas E, Verma IC Prenatal diagnosis of b thal : Experience in developing country Prenatal Diagnosis 1998Jan; 18(1): 1- 7	415 pregnancies	Use ARMS for prenatal diagnosis of B thal in 415 pregnancies of 360 women Confirm diagnosis in 98.3% and complete diagnosis was possible in 94.2% ARMS provides an inexpensive, robust and non isotopic for prenatl diagnosis of B thal in India with recommendation for establishing a prenatal diagnostic service in developing	
23	Sivera P et al Feasibility of molecular diagnosis of alpha thalassaemia in the evalution of microtosis Haematologica 1997 Sep-Oct; 82(5): 592-3		PCR is a useful in the diagnosis of alpha-Thalassemia, being able to identify carrier status in 82% patients with microcytosis, not related to iron deficiency or Beta-Thalassemia	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
24	Tongsong T. Chanprapaph P. et al. Cordocentesis at 16-24 weeks of gestation : experience of 1320 cases. Prenat Diagn 2000 Mar;20(3):224-8. SAFETY	Prospective study. 320 singleton pregnancies at risk.	Study to assess the safety and efficacy of diagnostic Cordocentesis at mid-pregnancy. 1 320 singleton pregnancies with no obvious congenital anomalies, a gestational age of 16-24 weeks. The maternal blood contamination rate was higher when the cord insertion was targeted. Procedure related complications included transient bleeding at puncture site (20.2%), transient fetal bradycardia (4.3%), chrioamnionitis (2 cases), and cord hematoma (1 case). Of 1281 successful cases, 184 fetuses had severe disease. The total fetal loss rate was 3.2% and the procedure related loss was 1%. The other obstetric complications were comparable with those in the general population. We concluded that cordocentesis at mid-pregnancy is a useful, relatively safe, and effective procedure for prenatal diagnosis.	
EFFECTIVENESS OF SCREENING PROGRAMMES				
1.	Cao A. et al Results of programmes for antenatal detection of thalassemia in reducing the incidence of the disorder. Blood Rev 1987 Sep;1(3):169-76.	Review	The characteristics and the effectiveness of programmes design to prevent beta-thalassemia major present in high frequency in several areas of the world such as Cyprus, Greece and Sardinia are reviewed. All these programmes are based on heterozygote detection, counselling and feotal diagnosis. The target population for screening have been couples at marriage, conception or early pregnancy. Awareness of the problem and involvement of the population was achieved via mass-media or personal approaches through lectures or discussions. Parent's Association were consulted and have been actively involved. Information leaflets have been made available to prospective couples at several critical areas. Education on thalassemia was introduced into the school curriculumn. Counselling was based on a private interview at which the several options available were discussed with the individual carrier or the couple. Prenatal diagnosis was chosen by large majority of couples counselled./ All these programmes resulted in a decline of	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
			thalassemia major births by 50-97%. The reasons for residual cases were mostly lack of information, and, less frequently, misdiagnoses or refusal of fetal diagnoses.	
2.	Cao A. Ristaldi M. et al. The prevention of thalassemia in sardinia. Clin Genet 1989 Nov;36(5):277-85	Retrospective study. Target population Screening.	Review of characteristics and effectiveness of a program aimed at preventing homozygous beta-thalassemia in the sardinian population. The target population for screening were couples at marriage, conception or early pregnancy. Awareness of the problem and the involvement of the population were achieved via the mass media or by personal approaches through lectures or discussions. Parent's Associations were consulted and have made themselves available to prospective couples in several critical areas. Education on thalassemias was introduced into the school curriculumn. Counseling was based on private interviews at which several options available were discussed with the individual carrier or the couples. Prenatal diagnosis was chosen by large majority of couples counseled. The introduction of 1st. trimester diagnosis resulted in a striking increase of the acceptance rate from 93.2 to 99.1%. Prenatal diagnosis was carried out initially by fetal blood analysis and thereafter by trophoblast or amniocyte DNA analysis. Direct detection of the mutation by oligonucleotide hybridization on agrose gel seperated DNA fragments or by dot-blot analysis with allelic specific oligonucleotide probes on enzymetically amplified DNA was used. This program resulted in a decline in thalassemia major births of 90%. The reasons for residual cases were mostly lack of information and, less frequently, misdiagnoses or refusal of fetal diagnosis.	
3.	Cao A. Rosatelli C. et al. Prenatal diagnosis and screening of hemoglobinopathies.	Review	This paper reviews the most important aspects of carrier detection procedures, genetic counselling, population screening and prenatal diagnosis of the thalassemias and sickle-cell disease. Carrier detection can be made retrospectively, following the birth of an affected child, or	

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	Baillieres Clin Haematol 1998 Mar;11(1):215-38.		prospectively. Carrier detection and genetic counselling in at-risk populations for alpha-thalassemia and sickle-cell anemia is carried out mostly retrospectively. However, prospective carrier screening is ongoing in Cuba and Guadeloupe for sickle cell anemia and in a very limited way, in some South East Asian populations, for alpha-thalassemia. For beta-thalassemia, several programmes, based on carrier screening and counselling of couples at marriage, preconception or early pregnancy, are operating in several Mediterranean at-risk population. These programmes have been very effective, as indicated by increasing knowledge on thalassemia and its prevention by target population and by the marked decline of the incidence of thalassemia major. Carrier detection is carried out by hematological methods followed by mutation detection by DNA analysis. Prenatal diagnosis is accomplished by mutation analysis on PCR-amplified DNA from chorionic villi. Future prospects include automation of the process of mutation-detection, simplification of pre-conception and pre-implantation diagnosis and fetal diagnosis by analysis of fetal cells in maternal circulation.	
4.	Kor-anantakul O. Rattanaprueksachart R. et al. Prenatal diagnosis of thalassemia in Songklanagarind Hospital in Southern Thailand. South-east Asian J trop Med Public Health 1998 Dec;29(4):795-80	Prospective screening program. 5078 pregnant women.	A thalassemia screening program for pregnant women has been established in Sobgklanagarind Hospital since 1992. After genetic counselling, a total of 5078 pregnant women accepted entry into a screening program for thalassemia. Couples at risk who should receive prenatal diagnosis were 2.8%. Total cases who accepted prenatal diagnosis were 135. total clinical cases were 40 (29.6%) with achievement by prenatal diagnosis of 33 cases (82.5%). Genetic amniocentesis is the most acceptable method for prenatal diagnosis. 5 cases (12.5%) were misdiagnosed due to contamination of maternal blood cells in amniotic fluid cases. Questionable results were reported in 2 cases (5%). Abortion occurred in one case (0.7%). Improvement of surgical technique in prenatal diagnosis reduced the complications and contamination of	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
			maternal cells. This program shows the feasibility of prevention and control of thalassemia disease in Southern Thailand.	
5.	Lee C. So K. et al. Prevention of beta-thalassemia major by antenatal screening in Hong Kong. Pediatr Hematol Oncol 1998 May-Jun;15(3):249-54.	Retrospective study. 34 thalassemia major children.	The thalassemia are common in Hong Kong. One of the severe form, thalassemia major, has been prevented locally by antenatal screening since 1983. Yet new patients are still being diagnosed. Retrospective study of 34 severe beta-thalassemia syndromes were diagnosed in two major public hospitals between 1990 and 1996. They include one pair of identical twins and two pairs of siblings. 27 (79%) had homozygous beta-thalassemia and 7 (21%) had beta E thalassemia. All but 4 (12%) were transfusion dependent. 55 (89%) parents had been evaluated for their thalassemia status. 48 had beta-thalassemia traits and seven were haemoglobin E carriers. The reasons for the birth of these children with severe beta-thalassemia syndromes were 1. late or no antenatal visit (n=8, 24.2%), including 3 cross-border deliveries in which the pregnant mothers came from mainland China to Hong Kong for confinement, 2.lack of maternal screenong (n=13,39.4%), 3. lack of paternal screening (n=2,6.1%). These findings suggest that several factors undermine the effectiveness of antenatal screening for prevention of thalassemias. Many medical practioners and the general public are still not aware of the screening procedures. The migration of population from mainland China to Hong Kong may result in the birth of many more children with beta-thalassemia major.	
6.	Mitchell J., Scriver C et al. 20 years outcome analysis of genetic screening programs for	Prospective study. 25 274 students.	The screening consisted of testing for carrier by NESTROFT in women with no risk and testing the husbands of the women with abnormal tests. A pregnancy in which both of the couple were carriers was considered at risk. Cordocentesis was performed in 554 pregnancy at risk. 252 from retrospective screening and 302 from prospective screening respectively, 60	

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	beta-thalassemia disease carriers in high schools. Am J Hun Genet 1996 Oct;59(4):793-8. 512		of 252 of the first group had severe thallasemia. In the prospective screening program of 12 680 women, 459 risk couples were identified, 302 pregnancies underwent cordocentesis and 53 (17.5%) had severe thalassemia. This strategy enable us to identify 113 cases of severe Thallasemia (Hb Bart 60, Beta-Thallasemia 53) from 554 cases at risk. In conclusion, the strategy proves valuable in the control of severe thalassemia. This extensive experience suggests the strategy be considered an effective way in the control of severe thalassemia in high prevalence area. -In Montreal programs for education, screening and counselling of senior-high-school students, in populations at high risk for beta-thalassemia have existed for > 20 years. 25 274 students were screened for beta-thalassemia, 693 carriers (frequency 1:36). All carriers identified in the high-school screening programme remembered their status, had their partner tested if they did not already known their were carrier couple, and took up the options for reproductive counselling/prenatal diagnosis. In Montreal, the current origin of all couples using prenatal diagnosis for beta-thallasemia disease is the corresponding genetic screening program, whereas, at the beginning of the programs, it was always because there was a history of an affected person in the family. Incidence of the disease has fallen by 90-95% over the 20 years. The rare cases are born (with 2 exceptions) outside the target communities or to non-screened couples.	
7.	Tongsong T, Chanprspaph P et al. Prenatal control of severe thalassemia – Chiang Mai strategy. Prenat Diagn 2000	Prospective and retrospective study. 7954 pregnancies.	Prenatal diagnosis strategy in preventing severe Thallasemia – more simple and inexpensive way; 1. Carrier identification of pregnancy at risk by retrospective screening (h/o known risk) and prospective screening for asymptomatic women. 2. Couple at risk offered genetic counselling. 3. Cordocentesis at 16-22 weeks / amniocentesis –	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
	Mar;20(3):229-34. Wanapirak C, Tuggapichitti A et al. Prenatal strategies for reducing severe thalassemia I pregnancy. Int J Gynaecol Obstet 1998 Mar;60(3):239-44	Prospective and retrospective study. 12 680 women.	polymerase chain reaction PCR (alpha-thallasemia). 4. Fetal blood analysis with high performance liquid chromatography (HPLC). 5. Counselling for termination of affected pregnancy. Couple at risk identified; 181 by prospective screening, 108 by retrospective screening, from 7954 pregnancies; 242 underwent cordocentesis, 108 from retrospective screening, 134 from prospective screening, 33 vs. 29 proven to have severe Thallasemia. The strategy identified all fetuses with severe Thallasemia with no false +ve among screen couples. In conclusion, the strategy proves to be highly effective in the control of severe thalassemia.	
	Schoen J. bachman P. et al. Comparing prenatal and neonatal diagnosis of haemoglobinopathies. Pediatrics 1993 Sep; 92(3):354-7.	Prospective study. 54 700 pregnant women.	To compare the result of prenatal and neonatal haemoglobinopathy screening, in Northern California, 54 700 pregnant women were screened for hamoglobinopathy. RESULTS: Of the 54 700 women screened, 1019 (1.9%) had haemoglobinopathy trait, and 81 women with at risk fetus were identified. Half the women with fetuses at risk for thalasssemia accepted prenatal-diagnosis of those whose fetuses were at risk of sickle-cell disease or other haemoglobinopathies, 30% accepted prenatal diagnosis. Of the 81 at-risk couples, 53 refused amniocentesis for definitive fetal diagnosis; only 28 (35%) accepted, all 4 women who are carrying a fetus with thalassemia major elect to terminate the pregnancy. Only 7 of the 21 cases of haemoglobinopathies were diagnosed prenatally; 14 were discovered neonatally. CONCLUSIONS: Prenatal screening was not found to be an ideal method of identifying hemoglobinopathies of the newborn in this large population. With cost-effectiveness a high priority in health care delivery, we believe that testing of newborns for haemoglobinopathy will continues to be the preferred screening method. A combined prenatal and neonatal program would offer the maximal benefit to patients by adding prenatal counselling, parental options, education,	

NO	Author, Title, Journal, Year,	Study design, Sample size, Follow Up	Outcomes & Characteristic	Grade & Comment
			and early complete diagnosis to neonatal screening.	
	Chadwick R et al Genetic Screening and Ethics Journal of Medicine and Philosophy		The extent of development of programme varies widely across Europe. Variations are due to the genetic disease patterns and the genetic services. Many country focus on screening programme to the pregnant woman and newborn. Newborn were screened for treatable disorder. Prenatal screening is for conditions which TOP may be offered. Established population screening programs for adults are thal carrier status in Cyprus, Greece and Italy. Social responses varies from acceptance to hostility. Legislation on genetic screening is important.	

EVIDENCE TABLE : SCREENING FOR BETA-THALASSEMIA TRAITS - SAFETY

No	Author, Title, Journal, Year,	Study Design, Sample Size, Follow-up	Outcomes & Characteristic	Grade & Comments
1.	Loukopoulos D. Fessas P. et al. Prevention of Thalassemia. Schweiz Med Wochenschr 1983 Oct 8; 113(40):1419-27	Retrospective study. 1088 cases.	Availability of prenatal diagnosis has contributed significantly to the acceptance by the public of the various screening programmes. The experience of the Greek group for prenatal diagnosis totals 1088 cases (June 1977 to Dec 1982). The number of couples at risk seeking the test prior to having any children is steadily increasing (prospective counselling). The efficacy of the overall prevention programme in Greece is reflected by the of that number of babies with Thalassemia major admitted for blood transfusion to the major Units of the country has decreased substantially in the last years. Routine methods used for prenatal diagnosis is biosynthesais of hemoglobin in samples of placental blood drawn by fetoscope. With increasing experience the procedure now is safe (< 1% obstetric complication) and the prediction accurate (no false diagnosis in the recent years). However, it is	

No	Author, Title, Journal, Year,	Study Design, Sample Size, Follow-up	Outcomes & Characteristic	Grade & Comments
			anticipated that conventional method will soon be replaced by studies of amniotic cell DNA, a procedure both safer and less traumatic, or by studies of amniotic cell DNA, which will allow prenatal diagnosis at an earlier stage of pregnancy.	
2.	Tongsong T. Chanprapaph P. et al. Cordocentesis at 16-24 weeks of gestation : experience of 1320 cases. Prenat Diagn 2000 Mar;20(3):224-8.	Prospective study. 320 singleton pregnancies at risk.	Study to assess the safety and efficacy of diagnostic Cordocentesis at mid-pregnancy. 1 320 singleton pregnancies with no obvious congenital anomalies, a gestational age of 16-24 weeks. The maternal blood contamination rate was higher when the cord insertion was targeted. Procedure related complications included transient bleeding at puncture site (20.2%), transient fetal bradycardia (4.3%), chrioamnionitis (2 cases), and cord hematoma (1 case). Of 1281 successful cases, 184 fetuses had severe disease. The total fetal loss rate was 3.2% and the procedure related loss was 1%. The other obstetric complications were comparable with those in the general population. We concluded that cordocentesis at mid-pregnancy is a useful, relatively safe, and effective procedure for prenatal diagnosis.	
3	Kor-anantakul O. Rattanaprueksachart R. et al. Prenatal diagnosis of thalassemia in Songklanagarind Hospital in Southern Thailand. South-east Asian J trop Med Public Health 1998 Dec;29(4):795-80	Prospective screening program. 5078 pregnant women.	A thalassemia screening program for pregnant women has been established in Sobgklanagarind Hospital since 1992. After genetic counselling, a total of 5078 pregnant women accepted entry into a screening program for thalassemia. Couples at risk who should receive prenatal diagnosis were 2.8%. Total cases who accepted prenatal diagnosis were 135. total clinical cases were 40 (29.6%) with achievement by prenatal diagnosis of 33 cases (82.5%). Genetic amniocentesis is the most acceptable method for prenatal diagnosis. 5 cases (12.5%) were misdiagnosed due to contamination of maternal blood cells in amniotic fluid cases. Questionable results were reported in 2 cases (5%). Abortion occurred in one case (0.7%). Improvement of surgical technique in prenatal diagnosis reduced the complications and contamination of maternal cells. This program shows the feasibility of	

No	Author, Title, Journal, Year,	Study Design, Sample Size, Follow-up	Outcomes & Characteristic	Grade & Comments
			prevention and control of thalassemia disease in Southern Thailand.	

EVIDENCE TABLE: SCREENING FOR BETA-THALASSEMIA TRAITS - COST

NO	Author, Title, Journal, Year,	Study Design, Sample Size, Follow - up	Outcomes & Characteristic	Grade & Comment
1.	Cronin E. Davies s et al. Organisation and cost-effectiveness of antenatal haemoglobinopathy screening and follow-up in a community-based programme. BJOG 2000 Apr; 107(4): 486-91.	Retrospective study. 2101 women booked at antenatal clinic.	A haematology department providing antenatal and neonatal screening programmes in Inner London. 2101 women booking at the antenatal clinic were screened. 1. Cost of identifying abnormal haemoglobin in mother (1 209) 2. Cost of identifying at-risk fetus before confirmation by prenatal diagnosis (12 455). 3. Cost of providing genetic information and counselling to mother with abnormal haemoglobin (1 109). 4. Programme saving from cases averted (161 000). Antenatal screening with follow-up counselling can be self-financing at most prevalence of haemoglobinopathy traits, with greater savings where a high proportion of the traits are beta-thalassemia. There is a net financial cost (11 350) only at prevalence below 2.5% of traits. Since there are other benefits is it likely that antenatal screening will be considered cost-effective even at quite low levels of trait prevalence.	
2.	Cronin E. Davis S et al. Costing model for neonatal screening and diagnosis of haemoglobinopathies. Arch Dis Child Fetal Neonatal Ed 1998 Nov; 79(3):F161-7.	Retrospective review. 47 948 babies screened. Period 1 year 1994.	A retrospective review made in a Haematology Dept. and sickle-cell and thalassemia centre, providing antenatal and neonatal screening programmes in Inner london, the cost for 47 948 babies screened, of whom 25 have significant haemoglobinopathy and 704 had haemoglobinopathy were assessed. Average cost/baby tested (isoelectric focusing and high power liquid chromotography) were 3.51 pounds / 3.83 pounds respectively; the cost per case of sickle cell disease identified (IEF/HPLC) was 6 738 pounds / 7 355 pounds; the cost per trait identified (IEF/HPLC) was 234 pounds / 255 pounds; the	

NO	Author, Title, Journal, Year,	Study Design, Sample Size, Follow - up	Outcomes & Characteristic	Grade & Comment
			cost per extra case of SCD and trait identified by universal programme varied. In conclusion, IEF and HPLC are very similar in terms of average cost/test. At 16 traits/1000 and 0.5 SCD/1000, there was no significant identification cost difference between universal and targeted programmes. Below this prevalence, a targeted program is cheaper, but likely to miss cases of SCD. If target programmes were 90-99% effective, universal programmes cease to be good value except at high prevalence. Greater use of prenatal diagnosis, resulting in termination, and therefore fewer affected births, reduces the cost effectiveness of universal screening programme. Screening services should aim to cover a screen population which will generate workload over 25 000 births per year, and preferably over 40 000 births per year.	
3.	de Silva S et all Thalassaemia in Sri Lanka: implication for the future health burden of Asian populations. Sri Lankan Thal. Study group. Lancet 2000 March 4 ; 355(9206):786-91,	Analysed blood samples from patients attending thal clinic in 9 hospitals. Defined different types of beta-thal by HPLC and DNA analysis. Sample size 703 patients and 1600 school children blood samples	This study estimated the burden of disease and requirement for its control by analysing Different type of thalassaemia gene and frequency. <u>Outcome:</u> 23 different mutation and about 70% of patient has thal. Phenotype. 15-50% patients were carriers. Conclusion: In Sri Lanka, interaction of 2 common beta-thal. Alleles will result in transfusion dependent disorder. 40% will have HbE/beta-thal of variable courses Management of thal will required 5% of total health budget.	
4.	Gessner B, Shaffer A et al. A cost-effectiveness	Cross-sectional study. Newborn haemoglobinopathy screening.	Comparing a policy of no screening to universal or targeted screening with selective follow-up only of infants with homozygous or compound heterozygous and to universal or targeted screening with complete follow-up, including follow-	

NO	Author, Title, Journal, Year,	Study Design, Sample Size, Follow - up	Outcomes & Characteristic	Grade & Comment
	evaluation of newborn haemoglobinopathy screening from the perspective of state health care systems. Early Hum Dev 1996 Jul; 45(3):257-75.		up of infants with clinically insignificant traits. Among the selective follow-up options, targeted screening would be the most cost-effective strategy for Alaska at a cost of $206 192 per death averted; by contrast, universal screening would prevent 50% more deaths at an incremental cost of $2 040 000 per death averted. Universal would be more cost-effective than targeted screening for several scenarios including a high prevalence, a low screening test cost, and a high cost per screen associated with racial targeting. Among the complete follow-up options, both targeted and universal screening would cost at least $200 000 per death averted over the range of all variables tested; the incremental cost of universal versus targeted screening would be at least $600 000 per death averted. In conclusion, each state should determine the most cost-effective option based on state-specific values for disease prevalence, test costs and racial targeting costs.	
5.	Ginsberg G. Tulchinsky T et all Cost benefit analysis of a National Thalassaemia prevention programme in Israel. Journal of Medical Screening 1998; 5(3): 120-6		Aim: Examining the cost and benefits of a national screening programme to prevent thal. In Israel. Lifetime health care cost of caring a person with thal major are $ 284,154. Cost included home infusion service, chelating agent, hospital stay,operation, outpatient visits, lab test, therapist and etc. Lost of earning , premature mortality account for $51,843 and $141,944 for each case. National screening programme cost $900,000 and prevent 13.4 homozygotes from being born at a cost of $67,369 for each birth prevented. Benefit cost ratio 6:1 Conclusion:	

NO	Author, Title, Journal, Year,	Study Design, Sample Size, Follow - up	Outcomes & Characteristic	Grade & Comment
			National screening has monetary benefits to the society and exceeds the screening programme cost.	
6.	Karnon J. Modell B. et al. Lifetime treatment costs of beta-thalassemia major. Clin Lab Haematol 1999 Dec; 21(6): 377-85.	Cost Model. Review.	Beta-thalassemia major is a serious genetic disorder, which results in a considerable increase in both acute and chronic morbidity, and mortality. Treatment is intensive and predictions of the costs incurred may aid health care planning. In this report, the costs to the health service providing treatment services for beta-thalassemia major patients, over the course of a lifetime, is calculated in order to assist resources allocation decisions. A cost model was developed, incorporating data from disparate sources. The undiscounted lifetime cost of treating a beta-thalassemia major patient was estimated to be pound 803 002, although when the costs were discounted at a rate of 6%, the life -time cost was reduced to pound 219 068. Within sensitivity analyses, the discounted cost ranged from approximately pound 188 000 to pound 226 000. This report may act as a guide to those involved in the planning of health care provision with regards to resources required to treat beta-thalassemia major patients. Such information may also be incorporated into the decision-making process for the provision of antenatal screening programmes for beta-thalassemia major.	
7.	Moatti J., Orsini A et al. Evaluationof cost-effectiveness of school screening for heriditary haemoglobin abnormalities: Prospective study in Bouches-du-Rhone. School screening for haemoglobinopathies.	Prospective study. 1977-1988. All pupils. 28 screening strategies comparison.	Cost effectiveness ratios could be improved by using a blood count if beta-thalassemia alone is screened. Further improvement of cost-effectiveness ratios could be obtained by limiting screening to ethnic groups most ‘at risk’; but such strategies raise ethical and acceptability issues.	

NO	Author, Title, Journal, Year,	Study Design, Sample Size, Follow - up	Outcomes & Characteristic	Grade & Comment
	Rev Epidemiol Sante Publique 1988; 36(6):395-408			
8.	Ostrowsky J. Scriver C. et. al. Cost-benefit analysis of a thalassemia disease prevention program Am J Public Health 1985 Jul; 75(7):732-6	Review	An economic perspective on prevention of beta-thalassemia disease by means of genetic screening and prenatal diagnosis in an established program in Quebec. The program screened 80% of at-risk persons in the high-risk communities, provides diagnosis to 75% of at-risk couples, and prevented 2/3 of new cases in the period of study. The additional costs of medical and public health resources, both incurred and avoided, resulting from use of these prevention services, were measured. The total direct cost per case prevented in the program is less than the cost for a single year of treatment for an individual with the disease. Sensitivity analysis accommodating demographic assumptions, participation rate, and discounting rates indicates that, even at rates of marriage, endogamy, and participation lower than observed in the current program, treatment costs will still exceed prevention costs when discounting is set at conventional rates of 4% and 8%. Cost-effectiveness of the program is confirmed.	
9.	Scriver C. Ostrowsky J. et al. Beta-thalassemia disease prevention : genetic medicine applied. Am J Hum Genet 1984 Sep;36(5):1024-38	Population screening. 6 748 persons. Over 3 years period.	Evaluation of a program for thalassemia-disease prevention, comprising of education, population screening for heterozygous, and reproductive counselling. Preprogram survey of 3 247 citizens in the high-risk communities (85% were high-school students) showed that 88% favoured a program but only 31% considered fetal diagnosis as an acceptable option. Screening in high school or before marriage was preferred by 56%. In a 25 months period (DEC'79 – Dec '82), 6 748 persons screened using MCV/HbA2 indices. The participation rate was 80% in the high school group. The frequency for beta-thalassemia heterozygosity was 4.7% with 10-fold variation among ethnic groups at risk. Survey of 60 carriers and 120 non-carriers after	

NO	Author, Title, Journal, Year,	Study Design, Sample Size, Follow - up	Outcomes & Characteristic	Grade & Comment
			screening high-school students (response rate 77%); most carriers told parents (95%) and friends (67%) the test result. Carrier would ascertain their spouse genotype (91%) and approved uniformly (95%) the high-school screening experience and its goal. In the 25-month interval, 11 fetal diagnosis performed (greater than 75% participation in target population) either by fetoscopy (haemoglobin in samples of placental blood drawn by fetoscope) and globin-chain analysis or by amniocentesis and genomic DNA analysis; two of three affected fetuses were aborted at parental request, there was one spontaneous abortion (after fetoscope), and seven live births. The at-risk couples claimed pregnancy would not be contemplated without the fetal-diagnosis option. Analysis of the economic costs of the program; cost per case prevented is approximately equal to $6 700, slightly less than cost- per- patient- treatment- year or about 4% of un-discounted treatment cost incurred in the first 25 years of life for an affected individual. These findings indicate: collective acceptance of program, appropriate attitudes among carriers, general acceptance and efficacy of fetal diagnosis, and global cost-effectiveness.	
10	Sangani B. Vas F. et al. Thalassemia in Bombay: the role of medical genetics in developing countries. Bull World Health Organ 1990;68(1):75-81.	Prospective study. 200 families with thalassemia children.	Study of 200 families with thalasemia children in Bombay showed that these childrens' treatment and needs place a significant, unaviodable and increasing demand on the public health services. At the same time, owning to the potential large number of patients and the difficulties of long-term management, the situation is characterised by evasions of the problem, failure of planning, no provision for prevention, and inadequate treatment leading to premature death among affected children. The burden on such families is greater in developing than in developed countries because, besides caring for the chronically sick child, their lives are dominated by the high costs of treatment, often amounting to 20-30% of the income for many families. Seven mothers with no healthy	

NO	Author, Title, Journal, Year,	Study Design, Sample Size, Follow - up	Outcomes & Characteristic	Grade & Comment
			child and 27 with only one healthy child had been sterilised. 90% of the reproductive-age couples felt that prenatal diagnosis was a necessity. Also, ignorance and prejudice in the community led to social isolation for forty families. The experience in Europe show that improved treatment is the key step in controlling thalassemia. A well-organised day-transfusion service is cost-effective, soon restoring the children to health and leading to increased optimism. The formation of associations by parents could mobilised community support for improved treatment and prevention, and increase public awareness of the problem. Thus, cost-effective management and prevention through screening, genetic counselling, and prenatal diagnosis are at least as important in the developing as in developed countries.	
10.	Plelan et al An analysis of relative costs and potential benefits of different policies for antenatal screening for beta Thalassaemia trait and variant hemoglobin J of Clinical Pathology 1999 Sept; 52 : 697-700		The cost of universal testing for variant Hbs and Beta-Thalassemia trait using HPLC, and selective testing using MCH as a screening test and HPLC as a diagnostic test found both costs to be comparable, as the higher reagent and instrument costs of HPLC were off-set by the lower labour costs	
11.	Geroge E Beta Thalassaemia in Malaysia Department of Pthology, Faculty of Medicine ,		The costs of repeated hospital admissions; blood transfusions, antibiotic therapy and expensive investigations, regular anti-chelating deferrosamine therapy; prolonged hormonal replacement therapy; treatment for complications such as insulin-dependent diabetes mellitus, cardiac failure, hepatitis and possibly HIV infections impose a substantial burden on the limited resources (George E, 1996).	

NO	Author, Title, Journal, Year,	Study Design, Sample Size, Follow - up	Outcomes & Characteristic	Grade & Comment
	HUKM 1996			

EVIDENCE TABLE : SCREENING FOR BETA-THALASSEMIA TRAITS - - SOCIAL ETHICAL& LEGAL

No	Author, Title, Journal, Year	Study Design, Sample Size, Follow up	Outcome & Characteristic	Grade & Comments
SOCIAL				
1.	Petrou M. Ward R. et al. Long term effect of prospective detection of high genetic risk on couples' reproductive life ; data for thalassemia. Prenat Diagn 2000 Jun; 20(6):469-74.	Retrospective study. 102 thalassemia couples.	Prospective risk detection with availability of prenatal diagnosis is the best service currently available for couples at high genetic risk. Here we described the long term effect of this service on the reproductive life of 102 couples at risk of thalassemia, whose risk was detected prospectively by carrier screening, who made use of prenatal diagnosis, and where the women is now over 40. Overall outcome for couples is described in terms of number of favourable versus unfavourable pregnancy outcomes.(a favourable pregnancy outcome = unaffected live-birth, or affected live-birth resulting from informed parental choice). The 102 couples had a total of 356 pregnancies, including 302 viable pregnancies, and 88% achieved a family unburdened by thalassemia. 68% of viable pregnancies had a favourable outcome, but only 43% of couples had a only favourable outcomes, and 26% lost two or more viable wanted pregnancies. When early losses are included 58% of pregnancies had a favourable outcomes, but only 30% of couples had only favourable outcomes, and 41% lost two or more pregnancies. Even with the best available service, at risk couples remain victims of chance, and a significant minority experience great difficulty in obtaining even one healthy child. Research is needed on approaches that may allow couples better control of reproductive outcomes.	
2.	Med S. Petrou M et al.	Prospective study.	88.7% - women with affected fetuses terminate pregnancy. (11.3 % - declined principally on religious grounds).	

No	Author, Title, Journal, Year	Study Design, Sample Size, Follow up	Outcome & Characteristic	Grade & Comments
	Prenatal diagnosis of beta-thalassemia in Pakistan – experience in a Muslim country. Prenat Diagn 2000 May; 20(5):378-83.	300 couples, over 31/2 years.	1 year post-screening – 72% couples with affected fetuses still know of availability of screening test, 2/3 had had pregnancy but only 56% go for second prenatal diagnosis. Reasons given cost of test, fear of undergoing test with some, no clear explanation given.	
3.	Zahed L, Dames J et al. Acceptance of first trimester prenatal diagnosis for haemoglobinopathies in Lebanon. Prenat Diagn 1997 May;1795):423-8.	Interview. 83 couples at risk.	Most families had poor education, low socio-economic status and more than half of couples not aware of their genetic risk. 59% couples definitely in favour of prenatal diagnosis, 23% were uncertain and 18% were opposed to such testing, because of religious conviction against termination of pregnancy. Another important factor that influence choice was the cost of the test. Issues that arise that importance of a control program adapted to particular populations, proper information and counselling, and the need for financial support.	
4.	Sangani B. Vas F. et al. Thalassemia in Bombay: the role of medical genetics in developing countries. Bull World Health Organ 1990;68(1):75-81.	Prospective study. 200 families with thalassemia children.	Study of 200 families with thalasemia children in Bombay showed that these childrens' treatment and needs place a significant, unaviodable and increasing demand on the public health services. At the same time, owning to the potential large number of patients and the difficulties of long-term management, the situation is characterised by evasions of the problem, failure of planning, no provision for prevention, and inadequate treatment leading to premature death among affected children. The burden on such families is greater in developing than in developed countries because, besides caring for the chronically sick child, their lives are dominated by the high costs of treatment, often amounting to 20-30% of the income for many families. Seven mothers with no healthy child and 27 with only one healthy child had been sterilised. 90% of the reproductive-age couples felt that prenatal	

No	Author, Title, Journal, Year	Study Design, Sample Size, Follow up	Outcome & Characteristic	Grade & Comments
			diagnosis was a necessity. Also, ignorance and prejudice in the community led to social isolation for forty families. The experience in Europe show that improved treatment is the key step in controlling thalassemia. A well-organised day-transfusion service is cost-effective, soon restoring the children to health and leading to increased optimism. The formation of associations by parents could mobilised community support for improved treatment and prevention, and increase public awareness of the problem. Thus, cost-effective management and prevention through screening, genetic counselling, and prenatal diagnosis are at least as important in the developing as in developed countries.	
5.	Ghanei M, Adibi P et al Pre-marriage prevention of thal: reporting a 10,000 case experience in Isfahan Public Health 1997 May; 111(3): 153-6	Jan 93 to Jan 96 10,000 people preparing for marriage were screened	Religious restrictions on abortion: prevention by this way is not possible. Iran has a large no. of thal major. Aim: to describe an alternative means to prevent birth of thal. children Methods: 1.CBC and HbA2 level measurement 2. High risk couples - refer for further consultation regarding the disease and the means of its prevention. 90% decided not marry no new cases of thal. were detected in the children of screened population. Conclusion: Where both members of the couple were trait positive, they decided not to marry rather than marry and use other or no methods of preventing thal. Genetic counselling centre can help to prevent most thal. cases.	
6	Ahmad S, Saleem M et al	300 couple requested test	Consultant Islamic scholars ruled that a pregnancy can be terminated before 120 days as gestation.	

No	Author, Title, Journal, Year	Study Design, Sample Size, Follow up	Outcome & Characteristic	Grade & Comments
	Prenatal Diagnosis of B-thal in Pakistan : Experience in a Muslim country Prenatal Diagnosis 2000 May; 20(5): 378-83 SOCIAL		Diagnosis made between 10-16 weeks gestation with 5% after 16 weeks Method: DNA analysis and ARMS Method. 88.7% had affected fetus. Agreed for TOP. 1 year after the screening service- 141 couples with affected child were interviewed. 72% knew about the service but only 56% used the prenatal diagnosis. Reasons for not testing: Fear and cost. Conclusion: Feasible and acceptable in Muslim country.	
7	Chadwick R et al Genetic Screening and Ethics Journal of Medicine and Philosophy		The extent of development of programme varies widely across Europe. Variations are due to the genetic disease patterns and the genetic services. Many country focus on screening programme to the pregnant woman and newborn. Newborn were screened for treatable disorder. Prenatal screening is for conditions which TOP may be offered. Established population screening programs for adults are thal carrier status in Cyprus, Greece and Italy. Social responses varies from acceptance to hostility. Legislation on genetic screening is important.	
ETHICAL				
1	Chadwick R et al Genetic Screening and Ethics Journal of Medicine and Philosophy		The extent of development of programme varies widely across Europe. Variations are due to the genetic disease patterns and the genetic services. Many country focus on screening programme to the pregnant woman and newborn. Newborn were screened for treatable disorder. Prenatal screening is for conditions which TOP may be offered. Established population screening programs for adults are thal carrier status in Cyprus, Greece and Italy.	

No	Author, Title, Journal, Year	Study Design, Sample Size, Follow up	Outcome & Characteristic	Grade & Comments
			Social responses varies from acceptance to hostility. Legislation on genetic screening is important.	
LEGAL				
1	Chadwick R et al Genetic Screening and Ethics Journal of Medicine and Philosophy		The extent of development of programme varies widely across Europe. Variations are due to the genetic disease patterns and the genetic services. Many country focus on screening programme to the pregnant woman and newborn. Newborn were screened for treatable disorder. Prenatal screening is for conditions which TOP may be offered. Established population screening programs for adults are thal carrier status in Cyprus, Greece and Italy. Social responses varies from acceptance to hostility. Legislation on genetic screening is important.	

Appendix 1

LEVELS OF EVIDENCE SCALE

Level	Strength of Evidence	Study Design
1	Good	Meta-analysis of RCT, Systematic reviews.
2	Good	Large sample of RCT
3	Good to fair	Small sample of RCT
4		Non-randomised controlled prospective trial
5	Fair	Non-randomised controlled prospective trial with historical control
6	Fair	Cohort studies
7	Poor	Case-control studies
8	Poor	Non-controlled clinical series, descriptive studies multi-centre
9	Poor	Expert committees, consensus, case reports, anecdotes

SOURCE: ADAPTED FROM CATALONIAN AGENCY FOR HEALTH TECHNOLOGY ASSESSMENT (CAHTA), SPAIN