1.1 Introduction:

Down's syndrome is the most common chromosomal disease among live born infants, with an incidence of 1 in 600. The syndrome is caused by trisomy of chromosome 21 in the vast majority of cases (95%), the rare reasons for this condition being unbalanced chromosome translocation and chromosomal mosaicism. Trisomy itself is a consequence of meiotic non disjunction of chromosome alleles, and in 80% it is maternal in origin. DS is associated with mental handicap, cardiac and gastrointestinal anomalies and vulnerability to infections and leukemia and later to Alzheimer-like dementia. (Simola 1998).

The syndrome was named after Dr. Langdon Down, a physician at London Hospital, who recognized in 1866 that the condition was congenital, dating from intrauterine life (Down 1866). Antenatal diagnosis became possible after the establishment of the method of cultivating fetal cells from amnion fluid for chromosome analysis. DS prevalence was known to increase strongly with advancing maternal age, and amniocentesis was therefore offered to women over 35 years of age (Steele & Breg 1966).

Fetal nuchal translucency (NT) was first introduced in 1992 as a potential ultrasound marker of fetal chromosomal defects in the first trimester. It measured the maximum thickness of subcutaneous translucency between the skin and the soft tissue overlying the cervical spine in a sagittal section of the ferns. A thickness of 3mm or more was found to be associated with a more than 10-fold increase in risk for chromosomal abnormality (Nicolaides et al. I 992).

Numerous studies have since been published to assess the application of fetal NT in the first trimester with maternal age in assessment of the risk of aneuploidy, in particular. trisomy2l (PandyaeraL, 1995a; Szaboetal. 1995; TaipaleetaL. 1997; EconomidesetaL, 1998; Hafner et al.. 1998; Pajkrt etal., 1998; Snijders eta1. 1998; Theodoropoulos et aL, 1998; Schwarzler etal., 1999; Brizot etaL, 2001; Gasiorek-

Wiens etaL, 2001; Wayda et aZ 2001: Zoppi et al., 2001; Wald et al., 2003). In general, using a combination of maternal age and NT thickness in the first trimester for screening of fetal Down's syndrome, a detection rate of 69% at a false positive rate of 5% was reported (Wald et al., 2003). A higher detection rate could be achieved when NT was combined with other biochemical markers. These include pregnancy associated plasma protein-A in the first trimester. n-human chorionic gonadotrophin. alpha fetoprotein. Unconjugated estriol and inhibin A in the second trimester (Wald et aL, 2003).

NT thickness increases with gestational age (Pandya et al 1995a; Braithwaiteeral. 1996).The position of fetal neck (Whitlow et al. 1998). Presence of nuchal cord (Schaefer eral.,1998) and size of ultrasound image (Herman et al.. 1998 Edwards et cil. 2003) have been reported to affect the thickness of NT. Adequate training and quality assurance are important to ensure the accuracy of measurement. A standard technique has been advocated to ensure the repeatability of NT measurement (Pandya, 1999).

Like other biochemical' parameters. Various biological variables have also been found to constitute a difference in the thickness of NT. The effects of ethnicity (Thilaganathan et al.1998; Spenceretal.. 2000a; Chenetal., 2002), fetal gender(Spencer etal. 2000b; Lam et al. 200 1 ; Larsen et al.. 2002). gravity and parity (Spencer et aL. 2000c) on NT thickness have been studied. However. These alterations were not large enough to r&ij1t adjustments in the screening programs which use NT as one of the markers. The method of conception has been found to have a significant impact on the false positive rate in the screening of Down's syndrome. A significant elevation in the human chorionic gonadotrophin level and a significant reduction in the alpha fetoprotein level in the maternal serum in the second trimester were found in pregnancies conceived after assisted reproduction. This resulted in a false positive rate much higher than expected (Barkai et al..1996;

Heinonen et al., 1996; Ribbert et al., 1996; Frishman et aL, 1997; Lam et al., 1999;Hui et al.. 2003). With this observation, studies have proposed the use of maternal age and NT alone as the method of screening in these pregnancies (Maymon & Shulman., 2002).

It would be important to know if the thickness of NT in pregnancies conceived after assisted reproduction technology is different from those conceived spontaneously.

Screening of Down's syndrome by biochemical markers in multiple pregnancies poses a difficulty in pinpointina the affected fetus. This problem may be avoided by using NT for screening of trisomy 2 1. Studies have demonstrated that NT' thickness among twins was similar in comparison with singletons (Pandya etal., 1995b; Sebire et al.. 1996).

With the utilization of assisted reproduction technology, the incidence of multiple Pregnancies have been increasing. This group of women is relatively older and, hence, carries a higher risk of bearing a chromosomally abnormal fetus. It would be necessary to further evaluate if the reference range of NT thickness in naturally conceived singleton pregnancies could be employed in screening of Down's syndrome in multiple pregnancies conceived after assisted reproduction technology (Maymon et al.. 1999)

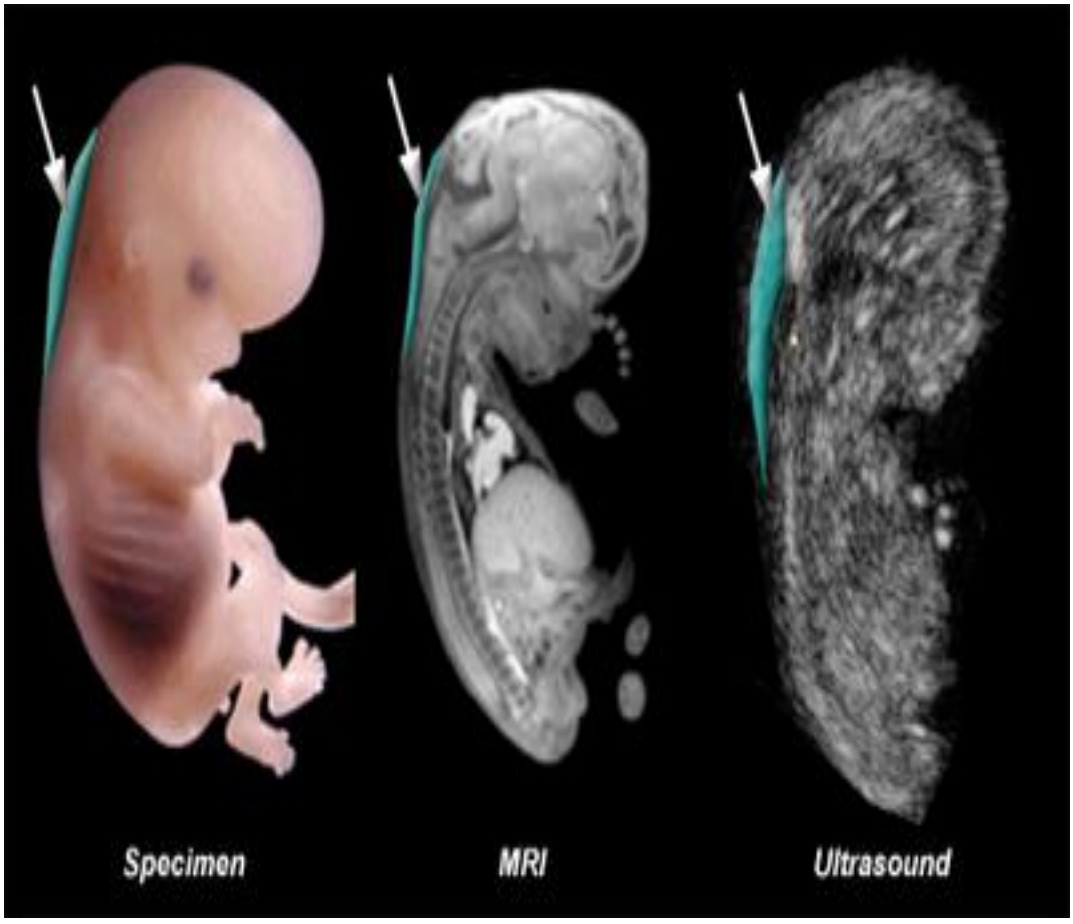


Figure (1.1); Shows represent three imaging modalities that

Identify early fetal development

The fetus on the left is an actual photo at 12 weeks. The fetus in the middle is from an MRI study and the fetus on the right is an ultrasound. The blue areas behind the neck represent the nuchal translucency that is measured during the first-trimester scan when evaluating the fetus for Down syndrome and other birth defects.

1.2 The Problem of the Study:

Nuchal Translucency (NT) changes may occur. In some cases, the measurements becomes too thick more than 3mm. these sign may lead to indicate of abnormal trisomy due to age over 35 years, it also can occurs during pregnancy before 35 years old when there is previous family chromosomal problems or no.

1.3 Objectives of the study:

1.3.1 General objectives:

- To evaluate the use of ultrasound measurements of fetal nuchal translucency (NT) obtained in a routine antenatal clinic setting in combination with appropriate biochemical markers as a first trimester screening test for done to screen for fetal anomalies**.**
- Antenatal Screening for Down's Syndrome.

1.3.2 Specific objectives:

- To measure Nuchal translucency from 10 weeks to 13weeks of gestation.
- To define the best fixed cutoff point of nuchal translucency and its accuracy in screening all fetal trisomy especially Down's syndrome .
- To investigate a method of screening for fetal trisomy's on the basis of maternal age and fetal nuchal translucency thickness at 10 to 13 weeks of gestation

1.4 Significance of the study:

- Incidence of chromosomal defects or abnormalities:

During the years 1995–99, nuchal translucency (NT) measurement was routinely offered to all women who had their dating scan in our unit. From the data collected, we calculated the 95th and 99th centiles of the NT for a given crown–rump length using regression analysis. The NT measurements were analyzed in relation to pregnancy outcome, especially with regards to miscarriage, intrauterine death and

diagnosis of fetal structural abnormalities, after excluding chromosomal abnormalities.

In the setting of routine antenatal screening, an increased NT measurement is a marker of a high-risk pregnancy even in karyotype normal fetuses. In addition, the increased incidence of structural abnormalities makes the close follow-up of these pregnancies imperative and should include specialized fetal echocardiograph.

1-5 previous studies:

- **Study** done by **Niemimaa M, Suonpää M, Perheentupa A, Seppälä M, Heinonen S, Laitinen** said that, 1. Screening for Down's syndrome in the first trimester by combining the measurement of fetal nuchal translucency and maternal serum ß-hCG and PAPP-A is an efficient method, also among unselected low risk women. Those centers which have established the NT screening should consider adopting the combined approach.

2. In IVF-pregnancies, ß-hCG is elevated in the first trimester due to unknown reasons, increasing the false positive rate. Serum screening is not recommended in these pregnancies.

3. The first trimester ultrasound screening based on measurement of nuchal translucency seems to decrease less the live born incidence of Down's children, compared with the second trimester maternal serum double screening, when the detection rate of the methods is similar. There is a concern that NT screening identifies preferentially those DS fetuses which are destined to miscarry.

- **A new study** (Screening for trisomy 21 in twin pregnancies in the first trimester: an update of the impact of chorionicity on maternal serum markers) done by **Kevin Spencer1,2*, Karl O. Kagan2,3** and **Kypros H** provides conclusive evidence that; Normative values have been generated to allow conversion of NT, free β-hCG and

PAPP-A to their MoM equivalents and correction factors have been determined to adjust for maternal and pregnancy characteristics for use in ethnic Chinese women undergoing first-trimester screening for aneuploidy.

● **Study** appears in **Prenatal Screening Unit, Clinical Biochemistry Department, King George Hospital, Goodmayes, IG3 8YB, UK**. (Screening for trisomy 21 in twin pregnancies in the first trimester: an update of the impact of chorionicity on maternal serum markers) **done** by **Kevin Spencer1,2*, Karl O. Kagan2,3 and Kypros H. Nicolaides2**. The researchers conclude that; In the first trimester, NT clearly has a much greater role to play in being able to provide an individual or fetal risk rather than biochemistry alone, which provides a pregnancy risk. Whilst NT can be used successfully to screen in twins with a similar detection rate and false positive rate to that in singleton pregnancies, the combination of both first-trimester NT and maternal serum biochemistry can improve the overall detection rate to around 80%.

● **Study** done by **Ksenija Gersak, Darija M. Strah and Maja Pohar-Perme (**Increased Fetal Nuchal Translucency Thickness and Normal Karyotype: Prenatal and Postnatal Outcome). They conclude that many couples enter any of the screening programs without an intricate understanding of the potential fetal and newborn complications. While it is reasonable for the future parents to consider normal karyotype as a "good" result, the healthcare professionals should counsel them that enlarged NT thickness is a strong marker for adverse pregnancy outcome, associated with miscarriage, intrauterine death, heart defects, numerous other structural abnormalities and genetic syndromes.

● **Study** appears in The **Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London.** (Fetal nuchal translucency: ultrasound screening for chromosomal defects in first trimester of pregnancy) **done by K H Nicolaides, G**

Azar, D Byrne, C Mansur, K Marksdone **They conclude that;** Fetal nuchal translucency >3 mm is a useful first trimester marker for fetal chromosomal abnormalities.

- **Other study** done by **Kypros H. Nicolai des*, Vi ctoria Heat h and Simon a Cicero(**Increased fetal nuchal translucency at 11–14 weeks). They conclude Increased NT is associated with a wide range of fetal defects and genetic syndromes and the prevalence of fetalabnormalities increases with NT: 3mm, 2.4%; 4 mm, 7.1%; 5 mm, 12.3% 6 mm, 16.7%; 7 mm, 35.6% (Souka et al., 1998). Furthermore, increased NT is associated with increased rates of miscarriage and perinatal death. However, it should be emphasized to the parents that increased NT per se does not constitute a fetal abnormality and, once chromosomal defects have been excluded, about 90% of pregnancies with fetal NT below 4.5–6.4 mm and 6.5 mm or more are about 80% and 45%, respectively.

- **Study** done by **Szabó et al;** (An ultrasound marker for fetal chromosomal abnormalities. The measurement of nuchal translucency in a South American population) which showed a high degree of accuracy in screening overall chromosomal abnormalities and even higher accuracy for trisomy 21. The best cutoff point obtained for nuchal translucency was values³ 2.5 mm.

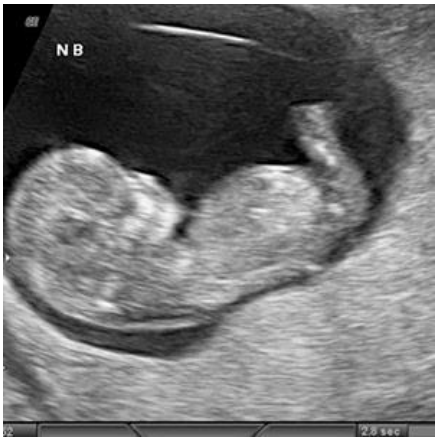
2. Literature Reviews:

2.1 Anatomy Nuchal Translucency:

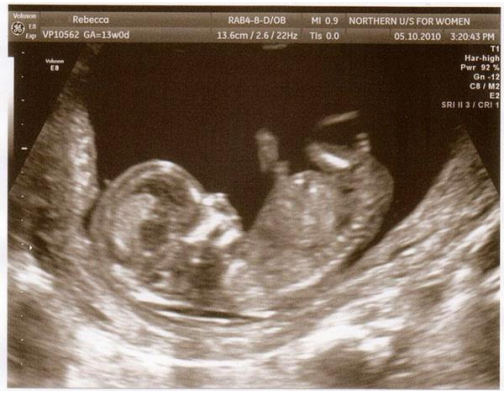
The nuchal translucency (subcutaneous) is fluid found at the back of fetus head and neck, between the skin and soft tissue just beneath the skin posterior to the cervical spine has to be measured. The thickness of this fluid can be precisely measured and this is called the nuchal translucency (or NT) measurement. Normally the amount of fluid is small, producing a thin NT measurement. We know that the amount of fluid can increase in the presence of certain conditions, producing a thicker NT measurement

1- 10 week fetus

2- 11 weeks fetus



3- 12 weeks fetus



4- 13 weeks fetus

Figure (2.1); (1-2-3-4) show Images of fetus during first trimester from 10 weeks to 14 weeks

2.2 Pathophysiology:

2.2.1 Down's Syndrome:

Down's syndrome, a classic chromosomal disorder resulting in mental retardation and severe congenital disorders, was the first medical condition to be associated with a chromosomal abnormality. With the incidence rate of one in every 750 live births, early detection through screening is imperative to help in prenatal diagnosis of Down's syndrome. This will provide the option of early termination of pregnancy and better obstetric care to the women with Down's syndrome pregnancies (Gardner and Sutherland, 2004; Roper and Reeves, 2006).

The Down's syndrome Screening Programme was started under the UK National Screening Committee (NSC). The UK NSC sets standards and oversees the implementation of screening programmes in England. The committee was set up in 1996. The recommended screening strategies from 2007 are the first trimester combined ultrasound and biochemical (CUB) screening, integrated testing and serum integrated testing. The Health Technology Assessment is currently reviewing two new strategies for screening, namely, repeated measure and cross trimester testing. These tests are expected to further improve the performance of Down's syndrome screening programmes in the period after 2010 (NHS Fetal Anomaly Screening Programme, 2008).

The earliest mention of this disorder was made by John Langdon Down in 1866. Down described this disorder as 'Mongolian Idiocy' in an essay classifying mental handicaps. However, the cause of the disorder remained unknown until 1959, when a French cytogeneticist, Jerome Lejeune, discovered trisomy 21 as the cause of this genetic abnormality. Subsequently, the condition was renamed as 'Down's Syndrome' in 1961, after John Langdon Down (Chudley and Chodirker, 2003).

2.2.2 Incidence Rate of Down's syndrome:

Down's syndrome, a classic chromosomal disorder, was the first medical condition to be associated with a chromosome abnormality in 1959 (Lejeune et al., 1959). In the absence of prenatal intervention, one in 750 live births in a typical population is affected by this chromosomal disorder (Gardner & Sutherland, 2004; Roper and Reeves, 2006). According to the Scottish Perinatal and Infant Mortality and Morbidity Report 2007, the rate of Down's syndrome in Scotland was 1.02 in 1000 births (1 in 980), during the period of 2002 to 2006 (Information Services Division NHS Scotland, 2008) and this lower incidence reflects the impact of screening and prenatal diagnosis. A large number of Down's syndrome pregnancies are sufficiently viable to survive to term (Cuckle, 2005). At conception, the frequency of Down's syndrome is much higher. Nearly 75% of the Down's syndrome fetuses identified during the first trimester, and about 50% of those identified during the second trimester are lost before the completion of the pregnancy term (Roper and Reeves, 2006). Advanced maternal age is the strongest risk factor linked to the cause of Down's syndrome pregnancies. The birth prevalence increases from 0.6 to 4.1 per 1,000 between the age of 15 and 45. This risk increases even more with a previous history of a Down's syndrome pregnancy (Cuckle, 2005).

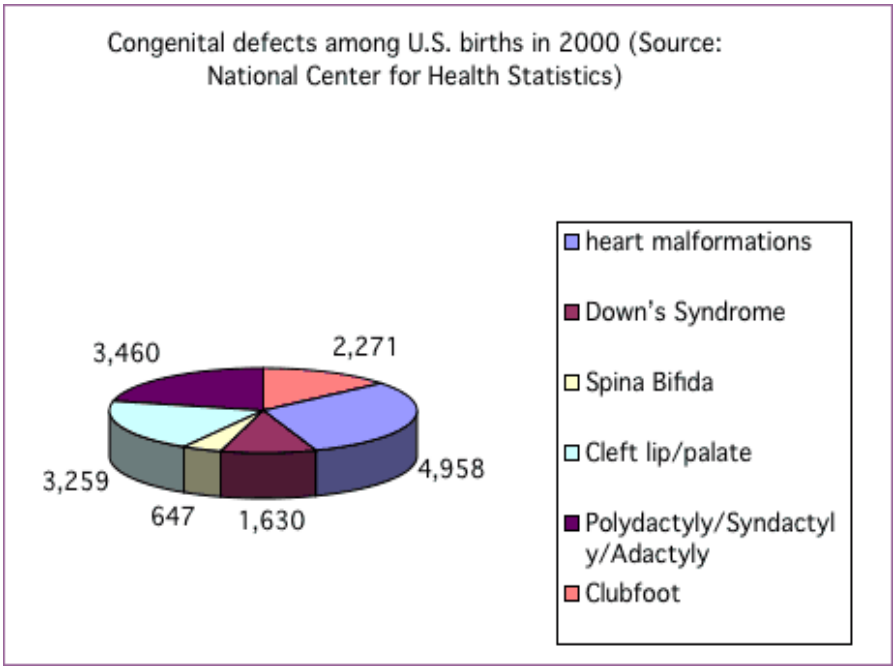


Figure (2.2). Shows Down's syndrome is one of the most common genetic conditions. Numbers are per 100,000 births.

2.2.3 Phenotype of Down's syndrome:

Down's syndrome is associated with variable phenotypes. However, mental retardation, neonatal hypotonia, small and hypocellular brain and minor facial dysmorphic features such as small nose, up-slanting palpebral fissures, speckling of iris (Brushfield spots), flat facial profile, low set ears, single palm crease, wide gap between the first and second toes and shortened fifth finger can be seen in almost all individuals with Down's syndrome (Korenberg *et al*., 1994).

Those with Down's syndrome also suffer from other congenital abnormalities such as heart defects and gastrointestinal abnormalities. A study conducted by Hayes *et al* (1997) in Dublin showed that heart defect is the most common

abnormality among children (found in 45.8%) with Down's syndrome followed by gastrointestinal disorders. This finding was consistent with another study conducted in Strasbourg by Stoll *et al* (1998). Other abnormalities such as urinary tract malformation, limb defects and congenital cataract have also been reported along with Alzheimer disease in those surviving beyond the age of 40 (Hayes et al., 1997; Stoll et al., 1998; Noble, 1998; Baliff and Mooney, 2003).Figure2.3

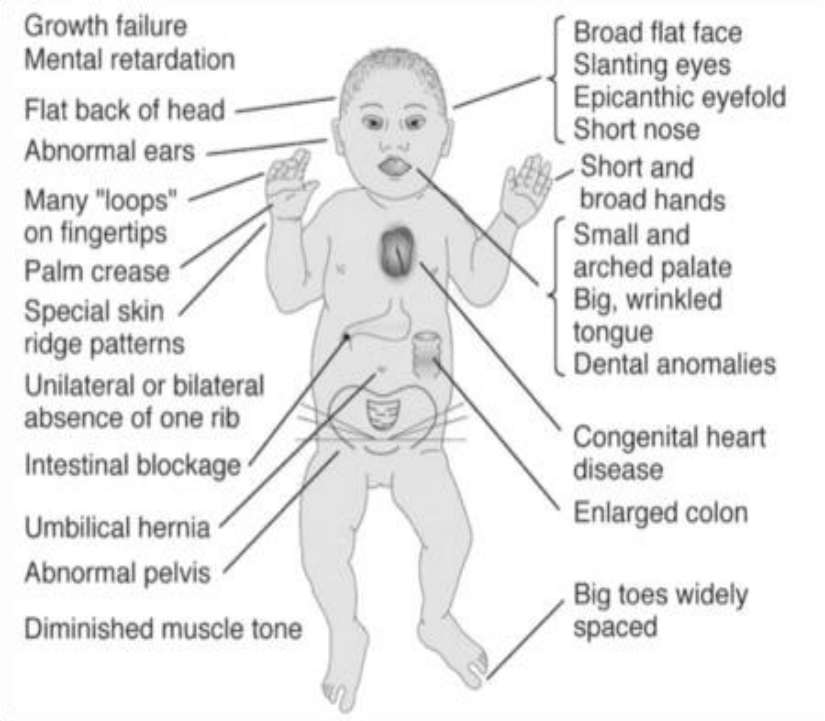


Figure (2.3). Shows Phenotype of Down's syndrome

2.2.4 Cytogenetic of Down's Syndrome:

Over 95% of Down's syndrome cases are caused by trisomy 21, where the cells in the body have three copies of chromosome 21 instead of the normal two. Studies have shown that non-disjunction at maternal meiosis 1 is the primary cause of most trisomy 21 cases (Robinson, 1977; Sherman et al., 1994; Noble, 1998). Non-disjunction occurs when homologous chromosomes fail to segregate symmetrically at cell division. This causes one daughter cell to have two copies of chromosome 21 and the other have none (Gardner & Sutherland, 2004). Figure 1.1

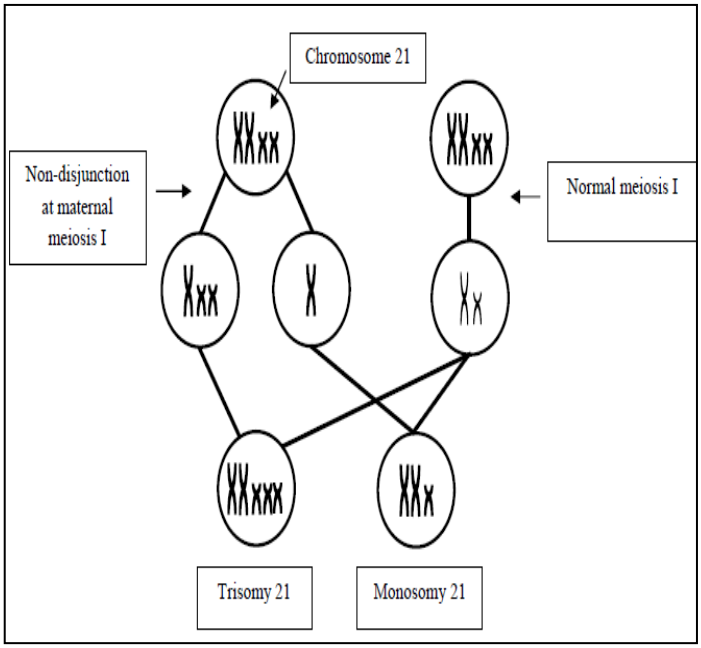


Figure (2.4). Shows the mechanism of non-disjunction in Trisomy 21

2.3 Maternal Age and Gestation:

The risk for many of the chromosomal defects increases with maternal age (Hecht and Hook, 1994). Additionally, because fetuses with chromosomal defects are more likely to die in utero than normal fetuses, the risk decreases with gestation. The rates of fetal death in trisomy 21 between 12 weeks (when NT screening is carried out) and term is 30% and between 16 weeks (when second trimester serum biochemistry screening is carried out) and term is 20% (Halliday et al., 1995; Snijders et al., 1995, 1999a; Morris et al., 1999).

With the development of prenatal screening, a need for maternal age-specific prevalence rates arose. A maternal age-specific rate schedule developed by Cuckle *et al* (1987) is widely employed for the purpose. The maternal age-specific risk schedule was developed by plotting a regression curve using the combined results of eight large, published surveys of Down's syndrome in live births. It was widely used in risk calculation and was embedded in many computer programmes used in routine screening. The widespread use of this rate schedule and the need for accurate maternal age-specific rates of Down's syndrome, led to further critical re-evaluations of this data (Hecht and Hook, 1994).

Subsequently, Hecht and Hook (1996) reported that the schedule in their study predicted higher rates than those predicted by Cuckle *et al* (1987), particularly in older women and proposed an alternate rate schedule. This finding was confirmed by Bray *et al* (1998) using meta-analysis of nine data sets to estimate maternal age-specific risk. In 1998, Cuckle investigated the effect of using different maternal age-specific prevalence curves on detection rate, for three second trimester screening protocols. Cuckle (1998) concluded that the inaccuracy caused by the use of different maternal age curves is unlikely to markedly influence the Down's syndrome screening result.

Pregnancies with Down's syndrome are likely to end in spontaneous fetal loss. Therefore, the risk of having pregnancy with Down's syndrome changes with gestational age. In 1999, Morris *et al* investigated the fetal loss rates in Down's syndrome pregnancies using data from National Down's syndrome Cytogenetics Register. Based on this study together with two other previous studies (Macintosh *et al*., 1995; Halliday *et al*., 1995), Morris *et al* (1999) reported that nearly 43% of pregnancies ended in a miscarriage or still birth between the time of CVS and term, and about 23% of miscarriages or still births occurred between the time of amniocentesis and term and 12% of births were stillborn or resulted in a neonatal death. A later study by Savva *et al* (2006) on the relationship between maternal age and the risk of spontaneous fetal loss in Down's syndrome pregnancies confirmed that the fetal loss rate in Down's syndrome pregnancies increases with maternal age.

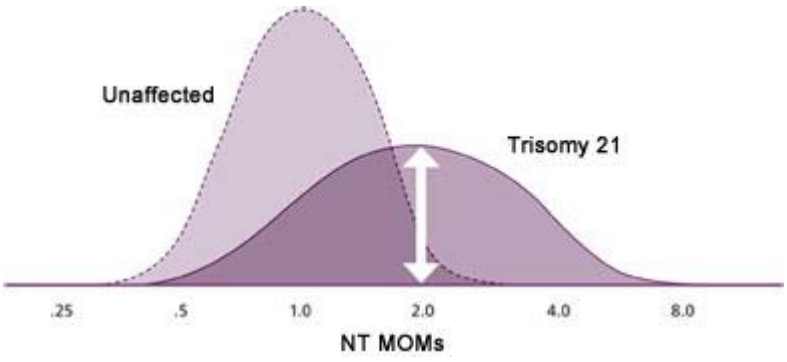


Figure (2.5). Shows NT MOMs in unaffected pregnancies and those with Trisomy 21 (Down's syndrome).

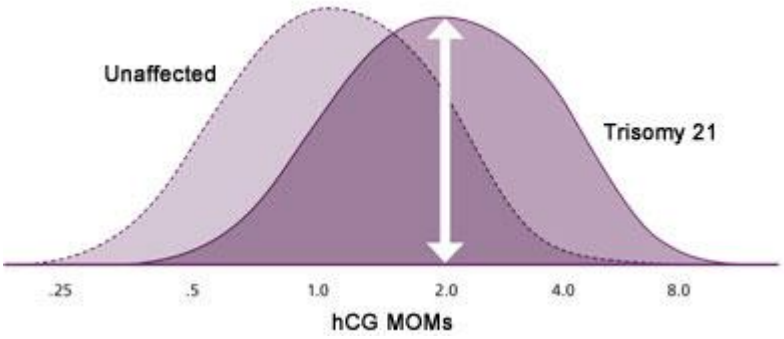


Figure (2. 6). Shows hCG MOMs in unaffected pregnancies and those with Trisomy 21 (Down's syndrome).

2.4 NT and other Chromosomal Defects:

In the Fetal Medicine Foundation Multicenter Project there were 325 cases with chromosomal abnormalities other than trisomy 21 (Snijders et al., 1998). In 71% of these, the fetal NT was above the 95th percentile of the normal range for CRL. Furthermore, in 78% of the pregnancies, the estimated risk for trisomy 21, based on maternal age and fetal NT, was more than 1 in 300. In addition to increased NT, there are other characteristic sonographic findings in these fetuses. In trisomy 18, there is early onset intrauterine growth Nuchal Translucency Screening Learn more about NT screening, an early, noninvasive option that can be exciting news for parents concerned about genetic disorders. Nuchal translucency screening, or NT screening, is an ultrasound test. It screens for Down's syndrome (trisomy 21, meaning an extra copy of chromosome 21) and other disorders that are caused by extra copies of chromosomes (trisomy 13, trisomy 18), as well as congenital heart defects. Restriction (IUGR), relative bradycardia and, in about 30% of the cases, there is an associated exomphalos (Sherrod et al., 1997). Trisomy 13 is characterized by fetal tachycardia, observed in about two-thirds of the cases, early-

onset IUGR, and holoprosencephaly or exomphalos in about 30% of the cases (Snijders et al., 1999b). Turner syndrome is characterized by fetal tachycardia, observed in about 50% of the cases, and early-onset IUGR (Sebire et al., 1998). In triploidy, there is early onset asymmetrical IUGR, relative bradycardia, holoprosencephaly, exomphalos or posterior fossa cyst in about 40% of cases, and molar changes in the placenta in about one-third of cases (Jauniaux et al., 1997).

Figure (2.7). Shows appearance of Trisomy 21investigation.

2.5 Diagnostic Methods:

2.5.1 Maternal Serum AFP (MSAFP) Technique:

Second trimester screening is performed between 15 and 20 weeks of gestation. In 1984, Maternal Serum AFP (MSAFP) Technique. was discovered to be a potential biochemical marker to identify pregnancies with increased risk of Down's syndrome and other trisomies (Merkatz et al., 1984). AFP is a 69kD protein that

belongs to the albuminoid family. AFP is synthesized by the yolk sac and the fetal liver (Powell et al., 1995, Seppala, 1975, Mizejewski, 2001).

During pregnancy, fetal AFP enters the maternal circulation via two possible pathways; transplacental diffusion and transamniotic membrane diffusion (Mizejewski, 2001). AFP concentration in the maternal circulation increases progressively to peak at 32 weeks (Macintosh and Chard, 1993).

According to several studies, a reduction in the maternal serum AFP level occurs in Down's syndrome pregnancies, in the second trimester (Merkatz et al., 1984; Cuckle et al.1984; Fuhrmann et al., 1984; Tabor et al., 1984). A study by Newby et al (1997) on biochemical markers and pathophysiology of Down's syndrome pregnancies indicated that the unchanged level of AFP in fetal liver homogenates and the significant elevation of AFP in placental tissue from Down's syndrome pregnancies suggest a possible transport defect specific to AFP which reduces the amount of AFP reaching the maternal circulation to about 75% of the level in unaffected pregnancies.

In 2002, Spencer et al studied the trend of marker median levels in Down's syndrome pregnancies between 6 and 20 weeks of gestation. Figure 1.2 illustrates the trend of multiple of the median (MoM) of AFP in Down's syndrome pregnancies between 6 and 20 weeks of gestation. The AFP measurement does not separate unaffected pregnancies from Down's syndrome pregnancies for gestational ages below 10 weeks. The optimum gestational age for AFP measurement for Down's syndrome screening is at approximately 16 weeks as there is the maximum separation at that gestational age (Spencer et al., 2002).

In the 1970s, screening for Down's syndrome was performed based on advanced maternal age alone. In 1987, Cuckle and co-workers estimated the risk of having a Down's syndrome pregnancy by combining maternal age and maternal serum AFP level. Cuckle et al (1987) reported that screening for Down's syndrome using both

maternal age and maternal serum AFP level was more efficient than using maternal age alone. For an example, using maternal age and AFP level, a detection rate of 28% with a false positive rate of 2.8% would be achieved for a risk cut-off of 1:200. Using maternal age alone, the same detection rate (28%) could be achieved with a higher false positive rate (4.3%) (Cuckle et al., 1987).

- **Technique of Doing AFP:**

The test is performed between the 8^{th} and 14^{th} weeks pregnancy, however it seems to be most accurate during the 16th to 18th week because levels of AFP vary during pregnancy so accurate pregnancy dating is imperative for more reliable screening results.

Blood is drawn from veins in the mother's arm and sent off to a laboratory for analysis. Results are usually returned between one and two weeks. Interpretation of the Result?

Alpha- fetoprotein (AFP) is found in both fetal serum and also amniotic fluid. This protein is produced early in gestation by the fetal yolk sac and then later in the liver and gastrointestinal tract. The true function of AFP is unknown. We do know that this protein's level increases and decreases during certain weeks of pregnancy which is why accurate pregnancy dating is crucial.

The AFP test is measuring high and low levels of alpha-fetoprotein. The results are combined with the mother's age and ethnicity in order to assess probabilities of potential genetic disorders.

High levels of AFP may suggest that the developing baby has a neural tube defect such as spina bifida or anencephaly. High levels of AFP may also suggest defects with the esophagus or a failure of your

baby's abdomen to close. However, the most common reason for elevated AFP levels is inaccurate dating of the pregnancy.

Low levels of AFP and abnormal levels of hCG and estriol may indicate that the developing baby has Trisomy 21(Down's syndrome), Trisomy 18 (Edwards Syndrome) or another type of chromosome abnormality.

2.5.2 Human Chorionic Gonadotropin (hCG):

In 1987, Bogart *et al* discovered an association between elevated second trimester human chorionic gonadotropin (hCG) levels and Down's syndrome pregnancies. Human chorionic gonadotropin is a glycoprotein hormone with a molecular weight of 36,000 to 46,000 daltons. Human chorionic gonadotropin is synthesized in the syncytiotrophoblast cells and composed of two subunits (alpha and beta). The alpha subunit has a structure similar to that of luteinizing hormone, follicle stimulating hormone and thyroid stimulating hormone.

Whereas, the beta subunit is a unique glycoprotein specific to hCG. In the circulation, hCG is mostly in the intact form and 0.3% to 4% exists as free beta human chorionic gonadotrophin (fβhCG) (Powell and Grudzinskas, 1995; Albertini *et al*., 1982; Macintosh and Chard, 1993). Spencer (1991) investigated the analytical and clinical performance of the measurement of second trimester fβhCG in pregnancies affected by Down's syndrome. The study demonstrated that fβhCG is elevated (0.99 MoM in unaffected, 2.06 MoM in Down's syndrome) in pregnancies affected by Down's syndrome. Studies by Newby *et al* (1997) also showed that hCG and fβhCG levels in second trimester placental tissue from Down's syndrome. pregnancies were higher than those in placental tissues from unaffected pregnancies. The similar changes of these markers both in the maternal serum and the placental tissue from Down's syndrome pregnancies suggest that the transport of these

markers from their site of synthesis to the maternal circulation is not affected in Down's syndrome pregnancies. Later studies of Spencer *et al* (2002) showed that optimum efficiency of screening using hCG can be achieved at 16 weeks of gestation. hCG level was found to be similar in both affected and unaffected pregnancies between 10 to 12 weeks of gestation (Figure 1.3)(Spencer *et al*., 2002).

2.5.3 Other studied serum markers:

Alpha-fetoprotein and unconjugated estriol are well-established markers of DS in the second trimester of pregnancy. AFP is also lowered in DS in the first trimester. A met analysis of 542 cases gave a mean MoM of 0.79 for AFP (Cuckle & Van Lith 1999). However, it has been shown that the standard deviation for AFP is increased by 20% compared to that reported in the second trimester. Therefore its contribution to the detection rate at a given false-positive rate will be lowered by the increase in overlap of the affected and unaffected distributions (Berry et al. 1995). Modeling suggests that adding AFP to the combination of PAPP-A and β-hCG increases the DR only by 2.0% (from 64.6% to 66.6%) (Cuckle & Van Lith 1999). Unconjugated estriol is also lowered in DS in the first trimester. A meta-analysis of 226 DS cases gave a mean MoM of 0.74. However, adding this marker to the combination of PAPP-A and β-hCG might increase the detection rate by only 4% (from 64.6% to 68.6%). (Cuckle & Van Lith 1999). Inhibin-A is reported to be increased in DS in the second trimester (Aitken et al. 1996, Cuckle et al. 1996) but its benefits as an additional serum marker are not accepted by all (Reynolds 2000). However, a recent report showed that quadruple screening yields a DR of 70% and performs better than triple or double screening (Wald et al. 2003). In the first trimester, the results are controversial. Some have reported a difference between affected and unaffected pregnancies (Wallace et al. 1995, Noble et al. 1997b) while others have not (Spencer et al. 2001). Inhibin-A seems to correlate strongly with β-hCG. Thus the sensitivity for trisomy 21 achieved through the combination of maternal serum

inhibin-A and ß-hCG is not significantly different from that achieved with ß-hCG alone. (Noble et al. 1997b).

2.5.4 Nuchal Translucency Screening:

Fetal nuchal translucency (NT) was first introduced in 1992 as a potential ultrasound marker of fetal chromosomal defects in the first trimester. It measured the maximum thickness of subcutaneous translucenc\ between the skin and the soft tissue overlying the cervical spine in a sagittal section ofthe ferns. A thickness of 3mm or more was found to be associated with a more than 10-fold increase in risk for chromosomal abnormality (Nicolaides et al. I 992).

Numerous studies have since been published to assess the application of fetal NT in the first trimester with maternal age in assessment of the risk of aneuploidy, in particular. trisomy2l (PandyaeraL, 1995a; Szaboetal. 1995; TaipaleetaL. 1997; EconomidesetaL, 1998; Hafner et al.. 1998; Pajkrt etal., 1998; Snijders eta1. 1998; Theodoropoulos et aL, 1998; Schwarzler etal., 1999; Brizot etaL, 2001; Gasiorek-Wiens etaL, 2001; Wayda etaZ 2001: Zoppi et al., 2001; Wald et al., 2003). In general, using a combination of maternal age and NT thickness in the first trimester for screening of fetal Down's syndrome, a detection rate of 69% at a false positive rate of 5% was reported (Wald et al., 2003). A higher detection rate could be achieved when NT was combined with other biochemical markers. These include pregnancy associated plasma protein-A in the first trimester. n-human chorionic gonadotrophin. Alpha fetoprotein. Unconjugated estriol and inhibin A in the second trimester (Wald et aL, 2003).NT thickness increases with gestational age (Pandya et aL 1995a; Braithwaiteeral. 1996).

The position of fetal neck (Whitlow et al. 1998). Presence of nuchal cord (Schaefer et al.1998) and size of ultrasound image (Herman et al.. 1998 Edwards et al. 2003) have been reported to affect the thickness of NT. Adequate training and quality assurance are

Important to ensure the accuracy of measurement. A standard technique has been Advocated to ensure the repeatability of NT measurement (Pandya, 1999).

Like other biochemical' parameters. Various biological variables have also been found to constitute a difference in the thickness of NT. The effects of ethnicity (Thilaganathan et al., 1998; Spenceretal.. 2000a; Chenetal., 2002), fetal gender(Spencer etal. 2000b; Lam etal. 200 1 ; Larsen et al.. 2002). Gravity and parity (Spencer et aL. 2000c) on NT thickness have been studied. However. These alterations were not large enough to r&ij1t adjustments in the screening programmes which use NT as one of the markers. The method of conception has been found to have a significant impact on the false positive rate in the screening of Down's syndrome. A significant elevation in the human chorionic gonadotrophin level and a significant reduction in the alpha fetoprotein level in the maternal serum in the second trimester were found in pregnancies conceived after assisted reproduction. This resulted in a false positive rate much higher than expected (Barkai et al.. 1996; Heinonen et al., 1996; Ribbert et al., 1996; Frishman et aL, 1997; Lam et al., 1999;Hui et al.. 2003). With this observation, studies have proposed the use ofmaternal age and NT alone as the method ofsereenirig in these pregnancies (Maymon & Shulman., 2002).

It would be important to know if the thickness of NT in pregnancies conceived after assisted reproduction technology is different from those conceived spontaneously. Screening of Down’s syndrome by biochemical markers in multiple pregnancies poses a difficulty in pinpointina the affected fetus. This problem may be avoided by using NT for screening of trisomy 21. Studies have demonstrated that NT' thickness among twins was similar in comparison with singletons (Pandya et al., 1995b; Sebire et al.. 1996; Maymon et al.. 1999a).

With the utilization of assisted reproduction technology, the incidence of multiple Pregnancies has been increasing. This group of women is relatively older and,

hence, carries a higher risk of bearing a chromosomally abnormal fetus. It would be necessary to further evaluate if the reference range of NT thickness in naturally conceived singleton pregnancies could be employed in screening of Down's syndrome in multiple pregnancies conceived after assisted reproduction technology.

- **Measurement technique:**

A strict mid sagittal section of the fetus in neutral position is essential in measurement of fetal NT. The image is magnified such that the fetus occupies as least 75% of the image. The head of the fetus should not be extended nor flexed. Care must be taken to distinguish between fetal sac and an-inion. The calipers are placed on the nuchal lines adjacent to the lucent area. The maximum thickness of the subcutaneous translucency between the skin and the soft tissue overlying the cervical spine is taken as the thickness of NT (Pandya, 1999).

- **Image size**

A magnified image with the fetus occupying at least three-quarters of the image was recommended (Pandya et aI.1999) as the standardized technique in measuring NT. The effect of image size has been studied in two series. A study from Herman et aI. (1998) showed that increased image magnification was associated with a statistically significant decrease in mean NT measurement, but the effect of image size was not considerable enough for a modification of caliper placement. The effect of image size was further confirmed by Edwards et aI. (2003). In this series, a variation of up to 29% in the mean NT 4measurement was found when the image magnification changed from 60% to 200%. lt is, therefore. Essential for screening centre to have an agreed standardization on image magnification for estimation of risk of Down' s syndrome.

●Caliper placement

As recommended by the Fetal Medicine Foundation on NT measurement, an on-to-On method with the calipers being placed on the nuchal lines just adjacent to the sonolucent area (Pandya, 1999) was advocated. The loss of repeatability of NT measurements could be largely accounted for by the placement of calipers (Pandya et al, I 995c). A study in Israel showed a significant difference of around I mm in NT measurement between onto- on and on-to-out (outer caliper being placed outside the skin line) methods (Herman. Et al, 2000). Which might have a considerable effect on the calculated risk of aneuploidy. This further illustrates the importance of employing a standardized measurement technique.

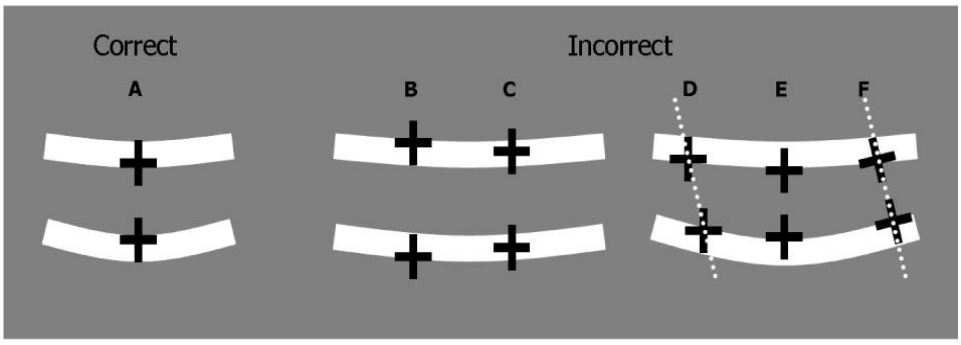


Figure (2. 8). Shows standardized measurement Technique

●Fetal neck position:

The thickness of NT was reported to differ markedly with changes in the degree of fetal neck flexion (Nicolaides et al.. 1992; Whitlow et aL, 1998). In a study with 196 cases in United Kingdom, the mean NT measurement taken in the mid-sagittal, extended position was 0.62 mm greater than that taken in the neutral position. The mean flexed NT was 040 mm less than the mean neutral NT. Repeatability of the measurements was more accurate with the fetal neck in the neutral position with the

repeatability coefficients being 0.48. 1.04 and 0.70 inneutral. Extended and flexed positions respectively (Whitlowetal.. 1998).

To help in capturing the best image in the mid-sagittal neutral position, a cine-loop Playback facility in the ultrasound machine would be useful.

●Fetal position

In addition to the position of fetal neck, the position of fetus has been speculated to have an effect on the thickness of NT due to the influence of gravity. This issue has been evaluated in a study in Netherlands. Which examined the mean NT thickness in 85 women with the fetuses in prone and supine positions. The mean NT thickness was comparable in these two positions, being 1.91 mm in supine fetuses and 1.93 mm in prone fetuses (de Graaf et al., 2000). Hence, gravity seems to have no influence on NT thickness.

●Nuchal cord

The umbilical cord may be mistaken as the NT when it is around the neck and the echogenic component of the cord is overlooked. The presence of nuchal cord s diagnosed when complete encirclement of the fetal neck by the umbilical cord was visualized on color Doppler ultrasound. This was found in 8.23% ofthe fetuses between 10 and 14 weeks of gestation. The presence of nuchal cord resulted in a mean of 0.8 mm being added to the ultrasound measurement of NT thickness. After the thickness of the cord was subtracted. The measurement of NT thickness did not different from those in the overall population in the study (Schaefer et al 1998). The use of color Doppler was advised to check for the presence of nuchal cord for appropriate adjustment to be made.

2.6 Factors affecting nuchal translucency measurement:

2.6.1 Gestational age:

NT measurement was found to increase significantly with gestation (Pandya et al., I 995a; Braithwaite et al.. i 996; Hsu et aL, 2003). It was positively correlated with crown rump length (Jou et aL, 2001). Using alog-linear model, NT was reported to increase by around 1 7% per gestational week (Schuchter et aL, 1 998). Therefore the use of a single threshold NT thickness throughout the first trimester was inappropriate. A gestational age dependent Cut off point should be adopted (Fajkrt et al. 1995; Braithwaite et al., 1996). To adjust for the effect of gestational age.

The NT measurement could be expressed as multiples of Medians (MoM) for a given gestation. This was calculated by dividing the NT by the expected median NT in an unaffected population for that particular gestational age. The median MoM in fetuses affected by Down's syndrome was 1.96 (Wald etal. 2003).

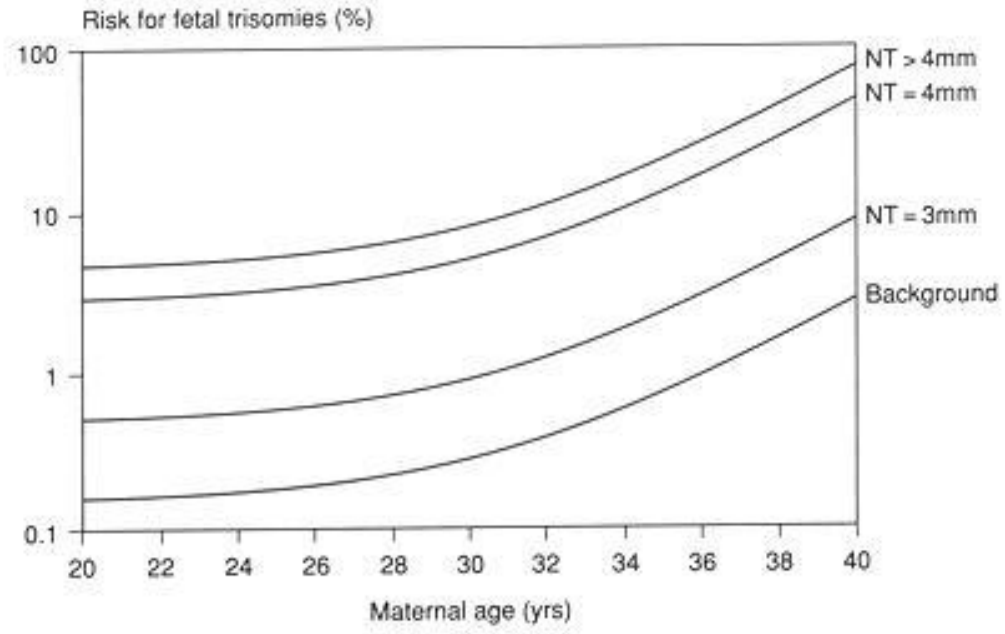


Figure (2.9*)*. Shows estimated risks of fetal trisomies at 10-14 weeks gestation on the basis of maternal age (background) alone and age plus nuchal fold thickness of 3mm, 4mm and >4mm. (Pandya et al 1995).

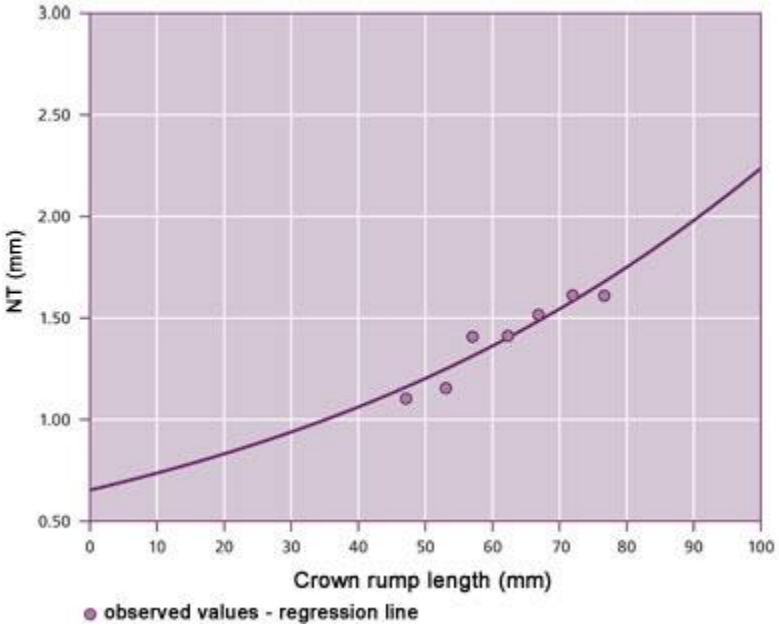


Figure (2.10). Shows monitoring of NT versus crown-rump length measurements from a sample cohort – recommended increment is 15-25% per week. This data set shows a 17.3% increase in median NT per week.

2.6.2 Ethnicity:

The concentrations of serum markers in Down's syndrome were affected by the ethnic origin of the population (Vatt el al.. 1996; Spencer et al., 2000a). Significant variations in The NT among different ethnic groups were found (Thailaganathaneral., 1998; ChenetaL, 2002). The mean MoMs in Caucasians. Africans. Asians and Caribbeans were reported as 1.02, 0.97. 1.05 and 0.99 respectively. The mean NT inthe Africans was 0.02 MoM less than the Caribbeans and 0.08 MoM less than the Asians (Thailaganathan et aL. I 998). In another study from Hong Kong, the median NT MoMs in Chinese, Filipinos, Caucasians and other Asians (Indians. Pakistanis and Nepalese) were 1.01, 1.07, 0.98 and 0.96

Respectively. The thickness of NT in Filipinos was significantly higher than other ethnic groups (Chen et al., 2002). Though the differences were statistically significant. The magnitudes of these differences were small when compared with the intraobserver and interobserver variabilities of 0.54 mm and 0.62 min respectively (Pandya et al. I 995).

Using a cut off of l: 180 in estimating the risk of Down's syndrome using matenal age and NT, there was no statistically significant difference in the screen positive rates reported by Chen et al. (2002). There were 5% of the Chinese, 4.6% ofthe Caucasians, 5.6% of the Filipinos and 4.2% of the other Asians (Indians, Pakistanis and Nepalese) being classified as screen positive (Chen et aL, 2002). Hence, the screening performance using NT should be comparable even in a multiethnic population.

2.6.3 Fetal gender:

A higher false positive rate in the presence of female fetuses has been reported in second trimester serum screening (Spong et al., I 999). The effect of fetal gender on NT was first addressed by Spencer e al. (2000b). In both chromosomaily normal and trisomy fetuses, the fetal NT was about 3-4% lower in female fetuses than in male fetuses. Another study also reported a significantly lower NT MoM in female fetuses examined sonographically from 1 1 weeks onwards. The median NT MoMs for female and male fetuses were 0.98 and 1.03 respectively (Lam etal.. 2001). The femaleJmaleNTMoM ratio was 0.95 by Lam et aL (2001); 0.979 by Spencer et aL (2000b); 1.00 by Yaron et al. (2001) and 0.979 by Larsen et al. (2002). Determination of fetal sex is possible at I O-14 weeks of gestation (Efrat et al., 1999; Wlitlow et aL, 1999). However. the accuracy of fetal gender assignment by ultrasound depends on the gestational week as well. lt is difficult to be consistently connect for fetal gender assessment at the time of NT examination, which make a correction not feasible (Spencer et aL 2000b).

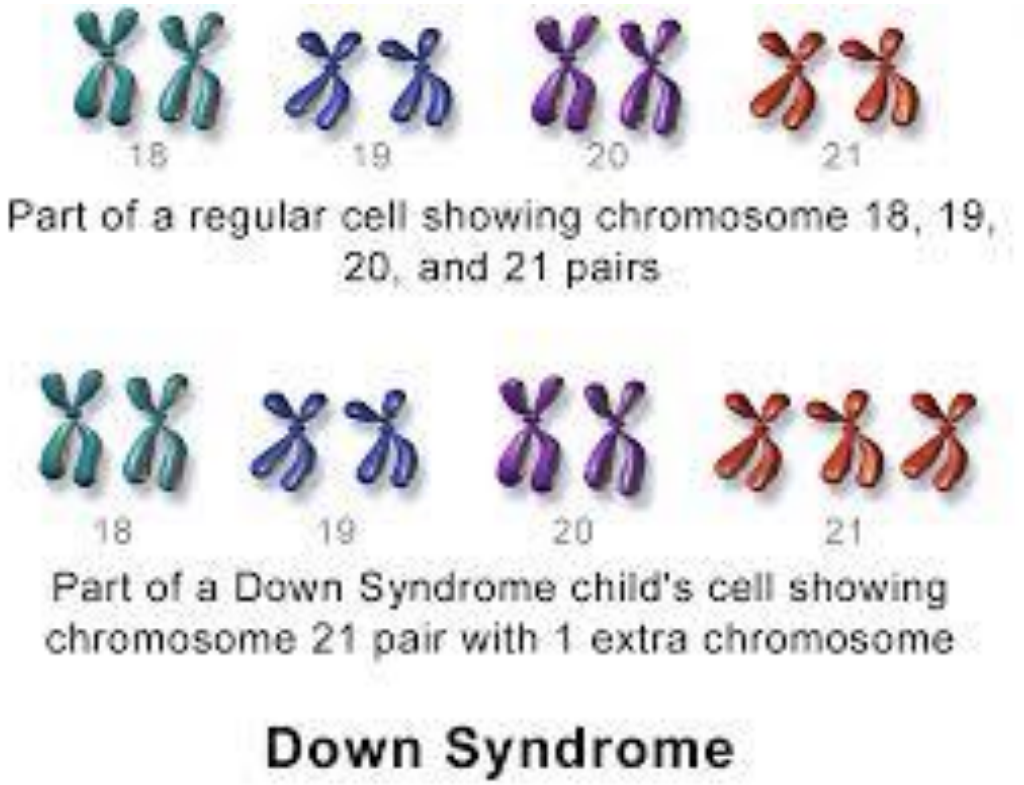
2.6.4 Gravity and parity:

Gravity and parity were shown to be associated with a small but progressive decrease in fetal NT. The NT MoM decreased by 1.3% for an increase in parity of one. But these changes were small and not statistically significant (Spencer et al., 2000e). Spencer also reported no correlation between NT in the first and second pregnancy in the same woman (Spencer 2002). The impact of gravity and parity on the screening performance of NT is likely negligible.

2.6.5 Mode of conception:

Studies have reported that the thickness of NT in pregnancies after in vitro fertilization was Comparable to the NT thickness in spontaneous pregnancies (Wojdemann et aL 200 1 ; Liao et aL. 200 1 ; Mavmon & Shulman 2OO2 Orlandi et al.. 2002). In pregnancies with embryos produced from intracytoplasmic sperm inj ection. the NT thickness was also found to be similar to that from naturally occurring pregnancies (Liao et aL. 200 1 ; Orlandi et aL 2002).

Pregnancies with frozen-thawed embryo transfers were not analyzed separately in these studies. From the degree of alterations in maternal serum human chorionic gonadotrophin and alpha fetoprotein concentrations in the second trimester, pregnancies from embryos with an addition of cryopreservation. Thawing and intracytoplasmic sperm injection Appeared to behave distinctly therefore, pregnancies from different methods of assisted reproduction should be regarded as separate entities in clinical management and research analysis (Hui et al., 2003).



Figure(2.11). Shows Down's Syndrome chromosome appearance

2.7 Relationship of Crown Rump length (CRL) and gestational age:

As crown rump length was not the only parameter utilized for gestational age estimation in the study, this was not used in the regression analysis. Instead the gestational age in terms of gestational days on the date of ultrasound examination was used. The relationship between crown rump length and gestational days for fetuses less than 13 weeks (91 days)in spontaneous pregnancies 'vas checked as an internal validation of the data The degree of correlation was examined using Pearson correlation analysis.

2.8 The use of Ultrasound in Diagnosis of Chromosomal Disorders:

Most of us look forward to ultrasounds during pregnancy: it's a chance to get a look at our babies, hopefully get a picture or a video, and maybe even find out if it's a boy or a girl. But we usually overlook the fact that the real reason for ultrasound (also called a sonogram) is for the doctor to make sure that everything is progressing properly in the pregnancy. And for most pregnancies, that's what they find.

2.8.1 Trisomy 21 (Down Syndrome):

Nuchal translucency thickness, choroid plexus cysts, echogenic intracardiac focus, echogenic bowel, renal pyelectasis, cystic hygroma, duodenal atresia, omphalocele, and failure to see the fetal nasal bone at 15-20 weeks.

2.8.2 Trisomy 18 (Edwards Syndrome):

Clenched hands, choroid plexus cysts, rocker bottom feet, delayed growth, heart defects such as VSD, ASD, and coarctation, kidney abnormalities, omphalocele, esophageal atresia, and polyhydramnios (excess amniotic fluid).

2.8.3 Trisomy 13 (Patau's Syndrome):

Cleft palate, polydactyly, small head, posterior heel prominence, delayed growth, heart defects such as VSD, ASD, PDA, and Dextrocardia, omphalocele, and myelomeningocele. For more explanation of these markers.

3. Materials and Methods:

3.1 Type of the Study:

Aquantitive descriptive study will be conducted with U/S in first trimester (10-13.6 weeks).

3.2 Area of the Study:

The study was conducted on Health care center that the patient was coming for follow up pregnancy period (Madinat Mohamed bin Zayed Health care center MMZC) in Abu Dhabi UAE.

3.3 Duration of the Study:

The study was carried out over duration of 12 month from April 2014 to April 2015.

3.4 Subject:

The data were collected from pregnant women with singleton pregnancies includes pregnant women aged from 20 years old and above to 45years old, different nationality, include two groups. Group (A) were pregnant women high positive test (AFP) and Group (B) pregnant women with normal level of (AFP).

- **Inclusion Criteria:**

Singleton Pregnant women attending routine antenatal clinics at 8th–14 weeks of gestation with the highest risks of having an affected pregnancy due to the result of AFP test.

All pregnant women should be offered the screening, were

- Women who have a family history of birth defects.
- Women who are 35 years or older.
- Women who used possible harmful medications or drugs during pregnancy.

- **Exclusion Criteria:**

Any pregnant women who have ectopic or molar pregnancies, confirm cardiac pulsation ,a tumor or liver disease in the woman and a normally elevated AFP in the fetus or woman (some people naturally have very high AFP) should be excluded from this study.

3.5 Data Collection:

Data collection according to work sheet (Appendix) includes all above variables data.

3.6 Data Analysis:

Data analysis by using SPSS .16.using significant tests like T test, frequencies and regression .and also the correlation between variables and prevalence of NT, correlations between, age , gender, weight, risk factors and prevalence of NT and diagnostic ultrasonography measurements of NT.

3.7 Ethical Consideration:

All results were taken from patients file after orally agreement from them and also after the agreement of the Head of MMZC Center and Medical Records Clerks.

3.8 Maternal serum AFP (MSAFP) Technique:

3.8.1 Packaging & Delivery:

Each Package contain cassettes (50 pcs /box 50pcs/bag (CORTEZ).

3.8.2 Specifications:

One Step AFP Test is a rapid test for the detection of Alpha Fetoprotein in serum as an aid in the diagnosis of primary Trisomy 21(Down syndrome), Trisomy 18 (Edwards Syndrome) or another type of chromosome abnormality

3.8.3 Specimen Collection:

For serum, collect blood into a container without anticoagulant. Allow the blood to clot and separate the serum from the clot. Use the serum for testing. If the specimen cannot be tested on the day of collection, store the serum specimen in a refrigerator or freezer. Bring the specimens to room temperature before testing. Do not freeze and thaw the specimen repeatedly.

3.8.4 Test procedure:

1- When you are ready to begin testing, open the sealed pouch by tearing along the notch. Remove the test from the pouch.

2- Draw 0.2ml (about 4 drops) sample into the pipette, and dispense it into the sample well on the cassette.

3- Wait 10-20 minutes and read results.

4- Do not read results after 30 minutes.

3.8.5 Interpretation of results:

A) Negative: Only one colored band appears on the control (C) region.

No apparent band on the test (T) region.

B) Positive: In addition to a pink colored control (C) band, a distinct pink colored band will also appear in the test (T) region. This indicates an AFP concentration of more than 25ng/mL.

C) If the test band is equal to or darker than the control band, it indicates that the AFP concentration of specimen has reached to or is greater than 400ng/mL. Please consult your physician to perform a much more

detailed exam.

D) Invalid: A total absence of color in both regions is an indication of procedure error and/or that the test reagent has deteriorated.

3.8.6 Storage and stability:

The test kits can be stored at room temperature (18 to 30°C) in the sealed p ouch to the date of expiration. The test kits should be kept away from direct sunlight, moisture and heat.

1- For in vitro diagnostic use only.

2. Do not use test kit beyond the expiry date.

3. The test device should not be reused.

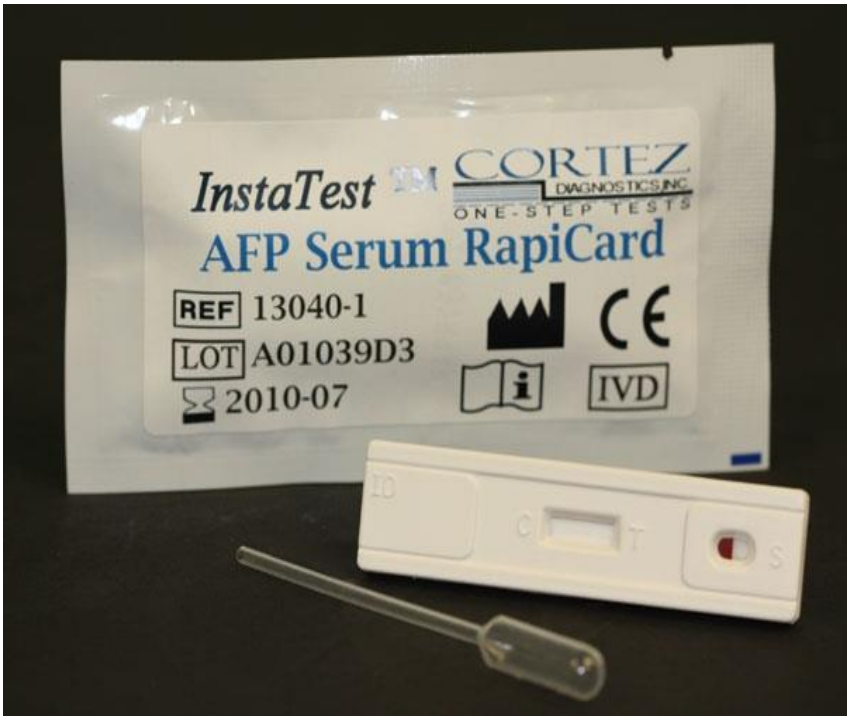


Figure (3.1). Shows AFP serum rapid card.

3.9 The NT Technique:

The Nuchal Translucency Scan is an ultrasound scan and is the first part of the Combined First Trimester Screening Test'. The second part is a specific blood test from the mother. All unborn babies have a collection of fluid found under the skin at the back of the baby's neck. The thickness of this fluid layer is called the 'Nuchal Translucency' and is measured with ultrasound. Ultrasound imaging uses soundwaves to take pictures. It does not use radiation. Nuchal Translucency is measured because research has shown a link between the thickness of the fluid and an increased risk of common chromosomal abnormalities such as Down's Syndrome. Factors such as the mothers' age, weight, blood test results, and the nuchal translucency details, are combined to give you a result. This is a screening test and will not tell you if your baby definitely has an abnormality but may help in Decide if further testing is needed.

3.9.1 Technique of doing NT Screening:-

- **Machine used:**

All patients scanned by GE Voluson 730 Ultrasound System. The GE Voluson 730 ultrasound features a state of the art user interface with easy to use on-screen menus, which will allow an operator to conduct scans more efficiently and accurately. With a 15" high resolution monitor, you'll be able to see ultra-clear results instantly, or they can be archived to CD, MOD or the 40GB hard drive. Among the other features of this model are tissue harmonic imaging, digital beamformer with 512 system processing channel technology and four active probe ports.

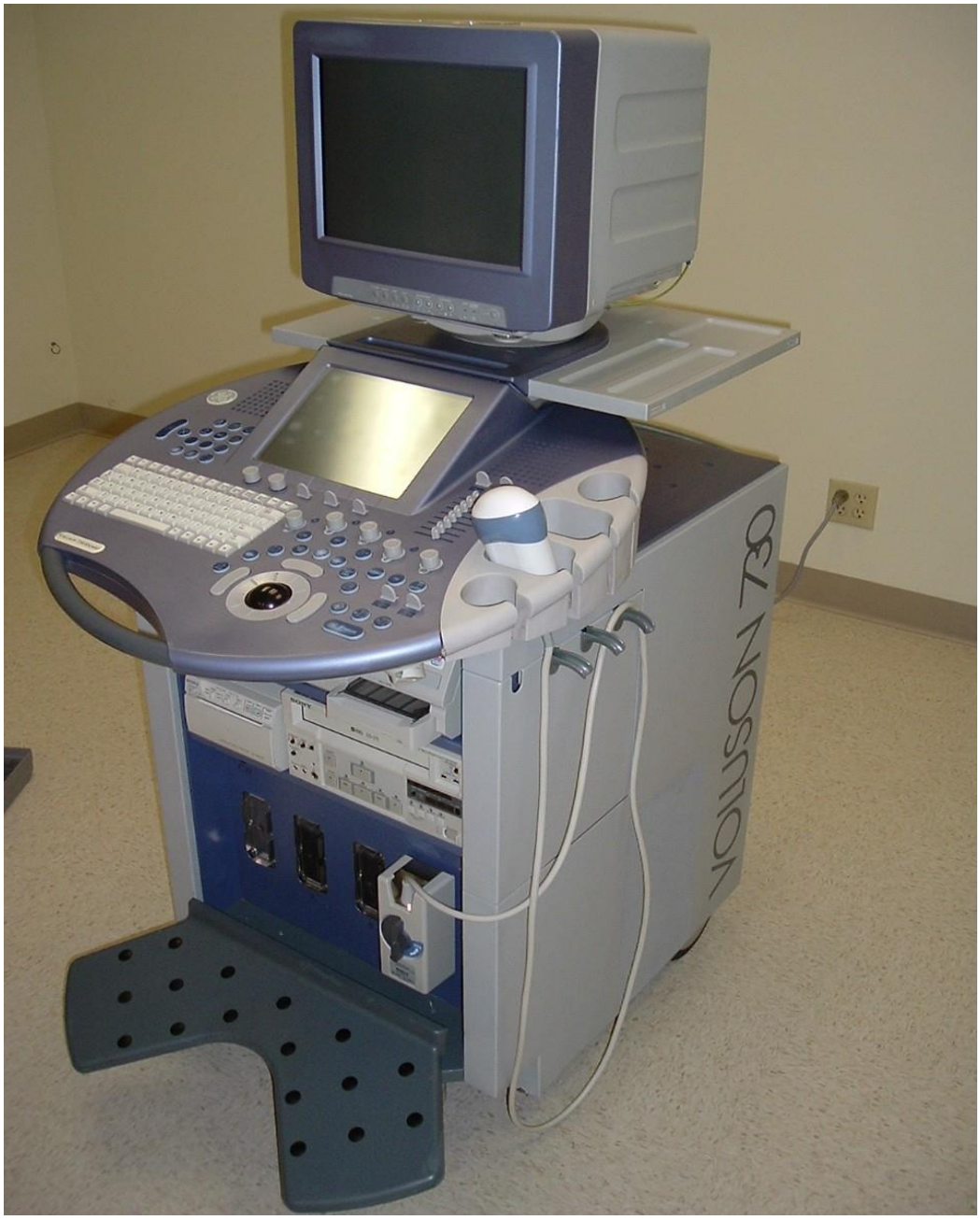


Figure (3.2). Shows GE Voluson 730 Ultrasound System.

- **Preparation before the Procedure:**

- A Nuchal Translucency scan is a painless procedure.
- No anesthetic is required.
- The medical imaging department will give you instructions on how to prepare for the scan.
- All pregnant women with their requests of blood test done one week before the procedure.
- wear a loose fitting, two-piece outfit because only your lower abdomen needs to prepare for the procedure

- **Preparations during the Procedure:**

1- The lights in the room will be dimmed so that the pictures can be seen more clearly on the display screen.
2- Pregnant women will lie in supine position and if difficult can take semi lateral decubitus position.
3- Ultrasound gel will be put onto the pregnant abdomen. The gel allows the probe to slide easily over the skin and helps to produce clearer pictures.
4- Ultrasound pictures are taken.
5- Once the scan is complete, the gel will be wiped off pregnant skin.
6- Depending on the procedure the ultrasound could take between 30 and 60 minutes. This time frame depends on the position and movement of the unborn baby.

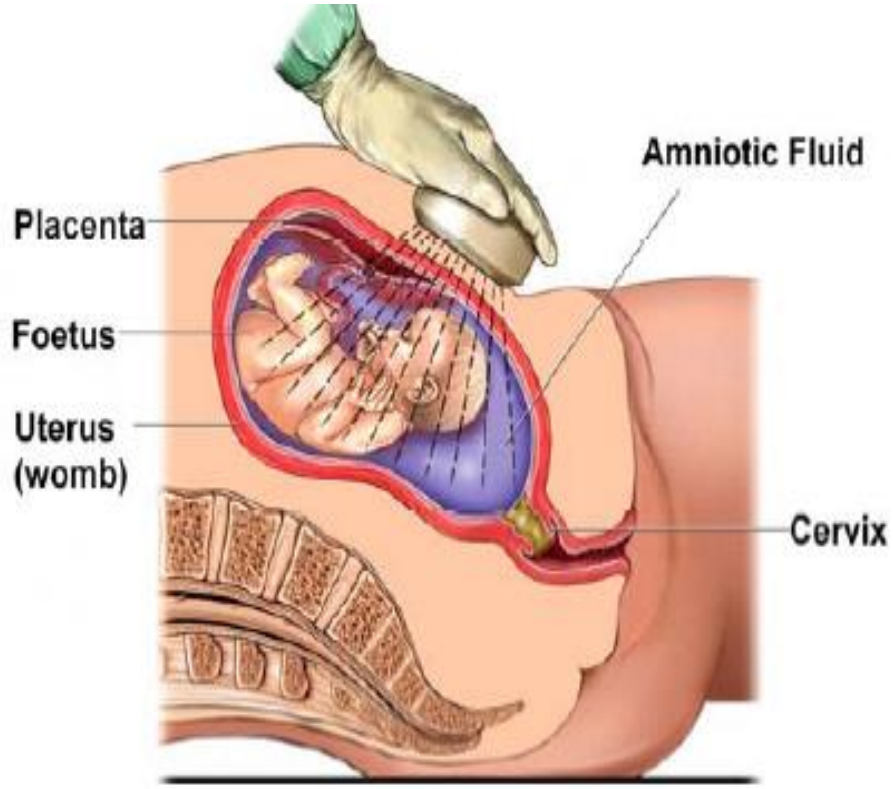


Figure (3. 3). Shows preparations during the Procedure of NT scanning.

- **Protocol for measurement of nuchal translucency:**

1. The gestational period must be 11 to 13 weeks and six days.
2. The fetal crown-rump length should be between 45 and 84 mm.
3. The magnification of the image should be such that the fetal head and thorax occupy the whole screen.
4. A mid-sagittal view of the face should be obtained. This is defined by the presence of the echogenic tip of the nose and rectangular shape of the palate anteriorly, the translucent diencephalon in the Centre and the nuchal membrane posteriorly. Minor deviations from the exact midline plane would cause non-visualization of the tip of the nose and visibility of the zygomatic process of the maxilla.

5. The fetus should be in a neutral position, with the head in line with the spine. When the fetal neck is hyperextended the measurement can be falsely increased and when the neck is flexed, the measurement can be falsely decreased.

6. Care must be taken to distinguish between fetal skin and amnion.

7. The widest part of translucency must always be measured.

8. Measurements should be taken with the inner border of the horizontal line of the aliper placed ON the line that defines the nuchal translucency thickness – the crossbar of the aliper should be such that it is hardly visible as it merges with the white line of the border, not in the nuchal fluid.

9. In magnifying the image (pre or post freeze zoom) it is important to turn the gain down. This avoids the mistake of placing the aliper on the fuzzy edge of the line which causes an underestimate of the nuchal measurement.

10. During the scan more than one measurement must be taken and the maximum one that meets all the above criteria should be recorded in the database.

11. The umbilical cord may be round the fetal neck in about 5% of cases and this finding may produce a falsely increased NT. In such cases, the measurements of NT above and below the cord are different and, in the calculation of risk, it is more appropriate to use the average of the two measurements.

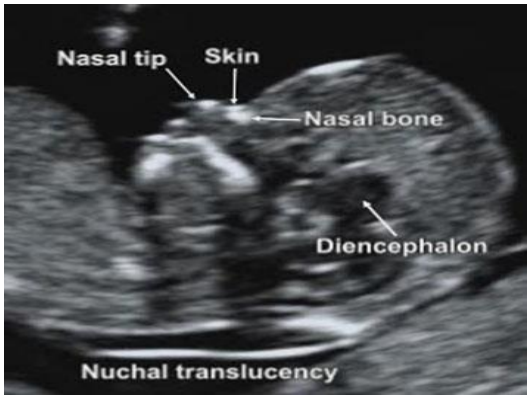


Figure (3.4). Shows protocol for measurement of nuchal translucency

• **Ultrasound Sonogram Markers of Down's Syndrome:**

There are several ultrasound markers for Down syndrome which can be seen on sonogram during pregnancy and which can possibly indicate an increased risk for the fetus having Down syndrome.

There are several ultrasound markers for Down syndrome which can be seen on sonogram during pregnancy and which can possibly indicate an increased risk for the fetus having Down's syndrome. Down's syndrome is genetic conditions associated with an extra #21 chromosome. Another works for Down's syndrome is also "Trisomy 21".

There are several ultrasound markers which can be seen on sonogram during pregnancy and which can possibly indicate an increased risk for the fetus having Down's syndrome.

●**These ultrasound markers include the following.**

A) Echogenic bowel:
B) Choroid plexus cysts
C) Structural anomalies (inc. cardiac)
D) Hypoplastic / absent midphalanx 5th Digit
E) Short femur
F) Wide space 1st-2nd toes
G) Short humerus
H) 2-vessel umbilical cord
I) Pyelectasis
J) Echogenic cardiac foci
K) Nuchal fold thickening >= 6 mm

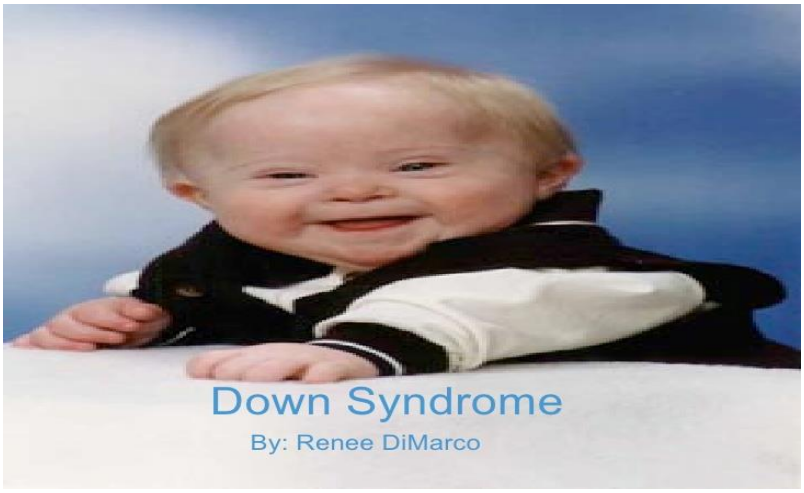


Figure (3.5). Shows Down's syndrome patient

4. Results:

4.1 Tables &Graphs:

Table (4.1): Shows Age Distribution of Experimental group.

Age Group	Total	Percent
19-25	11	7%
26-30	13	8%
31-35	45	28%
36-40	48	30%
41-45	43	27%
Total	160	100%

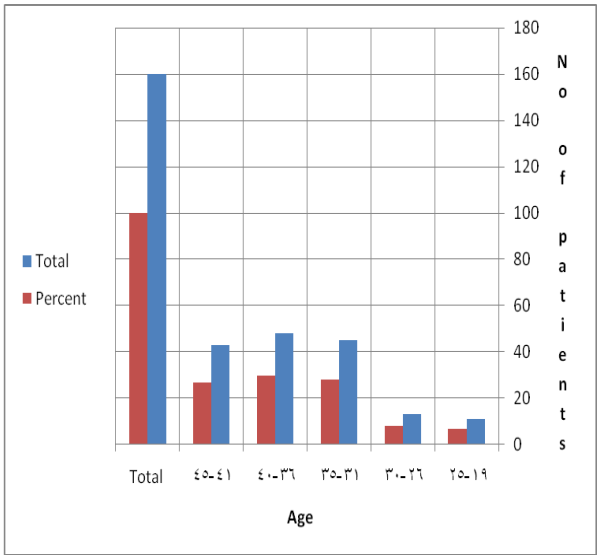


Figure (4.1): Age Distribution of experimental group.

Table (4.2): Shows age distribution of control group.

Age Group	Total	Percent
19-25	3	8
26-30	4	10
31-35	8	20
36-40	12	30
41-45	13	33
Total	40	100

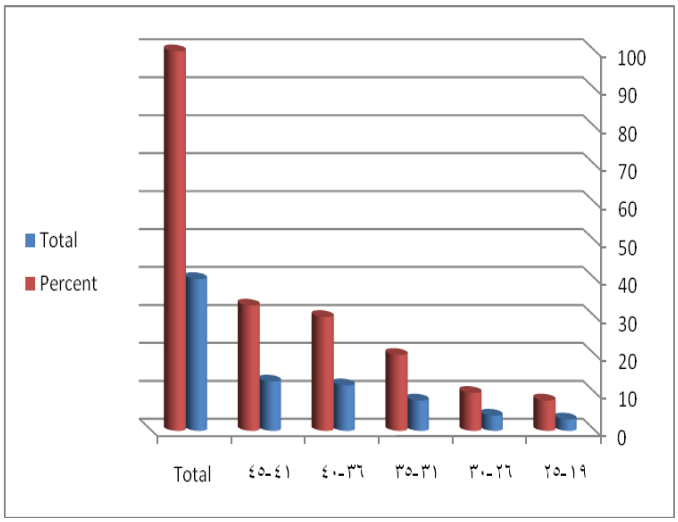


Figure (4.2): Shows age distribution of control group.

Table (4.3): Shows types of Trisomy

Types of Trisomy	Total	Percent
Trisomy 21	124	78%
Trisomy 18	22	14%
Trisomy 13	14	9%
Total	160	100%

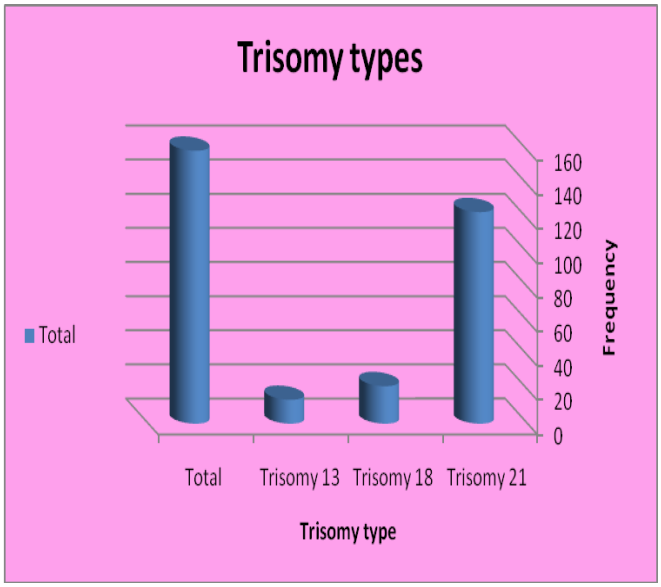


Figure (4.3): Shows types of Trisomy

Table (4.4): Shows outcome of pregnancies with respect to the NT thickness.

Outcome of pregnancies respect to NT thickness	≤ 3.4	3.5-4.4	4.5-5.4	5.5-6.4	≥ 6.5	Total	Percent
Delivery	51	14	4	0	0	69	43%
Miscarriage	28	3	8	1	0	40	25%
Intrauterine Death	20	1	0	2	1	24	15%
Termination	18	3	5	0	1	27	17%
Total	117	21	17	3	2	160	100%

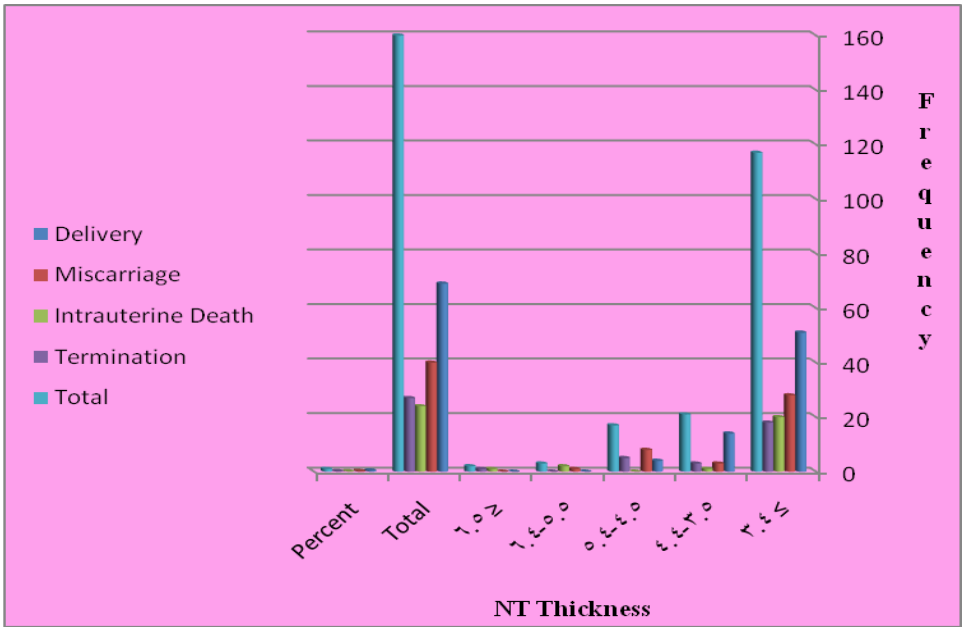


Figure (4.4): *Shows outcomes of pregnancies with respect to the NT thickness.*

Table(4.5): Shows clinical findings in live born infants with respect to the NT thickness.

Clinical Findings	N	Percent
Heart defects(VSD)	44	27.5%
Hydronephrosis	19	12%
Hydrocephalus	11	7%
Hemangioma	22	14%
Cleft lip /palate	45	28%
Micrognathia	11	7%
Craniosynostosis	8	5%
Total	160	100%

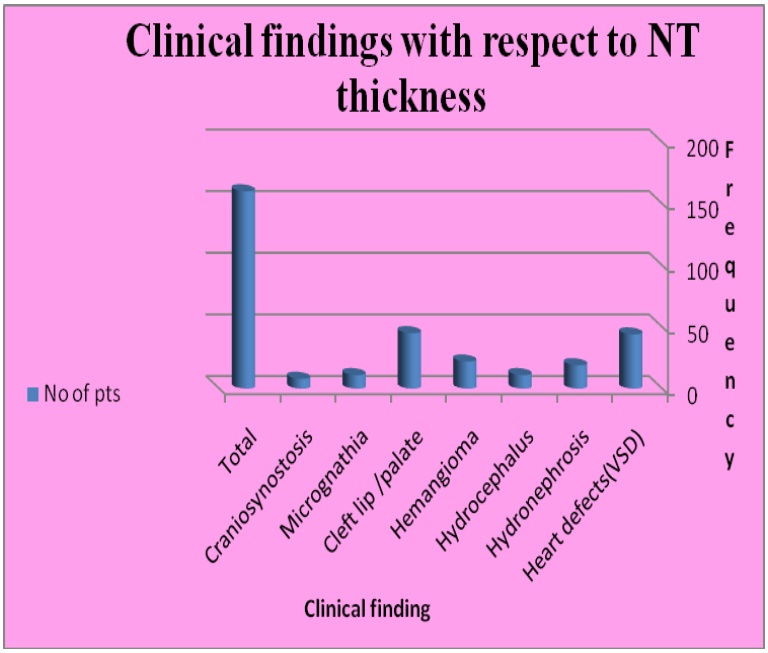


Figure (4. 5): Shows clinical findings in live born infants with respect to the NT thickness.

Table(4.6): Shows clinical findings in live born infants with respect to the NT thickness.

NT thickness	No of Pts	Percent
≤ 3.4	117	73%
3.5-4.4	21	13%
4.5-5.4	17	11%
5.5-6.4	3	2%
≥ 6.5	2	1%
Total	160	100%

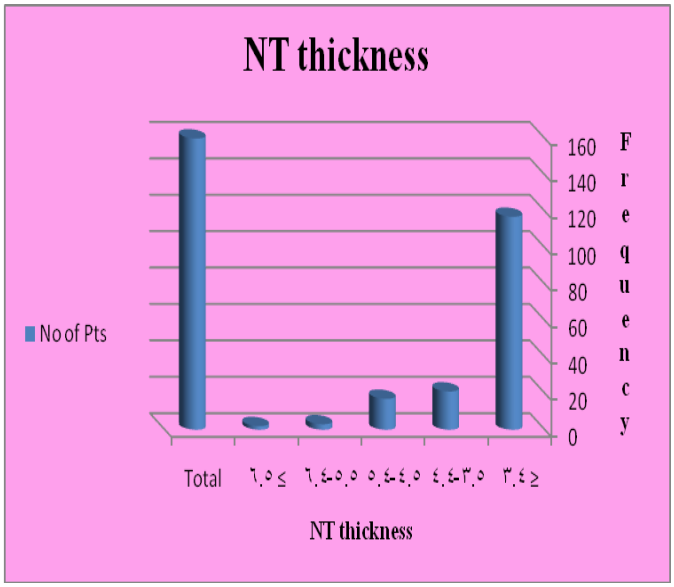


Figure (4.6): Shows clinical findings in live born infants with respect to the NT thickness.

5.1 Discussion:

The data was collected from trans abdominal scanning of 200 pregnant women with singleton pregnancies aged from 20 years old and above to 45years old, different nationality, include two groups. Group (A) were pregnant women high positive test (AFP) and Group (B) pregnant women with normal level of (AFP). The results of this demonstrate that fetal NT thickness increases with CRL and are compatible with those of previous reports **(Antolin et al 2001, Canick et aland Caughey et al).**

Table (4.1); Shows age distribution of pregnant women in experimental group recognized that many are getting markers of trisomys.

In **table (4.2);** The more affected age of study group between (35-40 years). similar result was achieved by **(Hecht and Hook, 2003** and **Kypros H et al)** which found that; the risk for many of the chromosomal defects increases with maternal age.

Table (4.3); Shows that trisomy 21 is more affect than trisomy 18 and trisomy 13 . In agreement to studies reported by **(Kevin Spencer et al., 1998)**.

In **Table (4.4);** On live born infants with respect of abnormalities heart defect is more recognition more than structural abnormalities and genetics syndromes. Similar result was achieved by (**Ksenija Gersak et al** and **Kypros H et al).**

Table (4.5); Which demonstrate the clinical findings most of live born infants with trisomy21 were affected by Heart defects (VSD). In agreement to studies reported by (**Ksenija Gersak et al** and **Kypros H et al).**

Also there was other study done by **Hayes et al (2001)** , found that; those with Down's syndrome also suffer from other congenital abnormalities such as heart defects and gastrointestinal abnormalities in Dublin showed that heart defect is the most common abnormality among children (found in 45.8%) with Down's syndrome followed by heart disease.

In **Table (4.6);** The increase of clinical findings in live born infants with respect to the NT thickness is comparable with **(Maymon et al.. 2005)**, he has Monitoring of NT versus crown-rump length measurements from a sample cohort – recommended increment is 15-25% per week. This data set shows a 17.3% increase of fetal abnormalities by increasing NT thickness. Therefore, in screening for chromosomal defects, the use for a fixed cut-off in fetal NT thickness is inappropriate, and each measurement should be examined according to the CRL **(Cole L, Rinne K et al 1999).** To express the relationship between NT measurement and gestational age **(Esterman A et al 1996).** This method is similar to that of biochemical markers used in second trimester Down screening **(Ferriman et al 1999).** Other studies asserted that the more accepted method is to base the cutoff on a progressive rise, using 95th percentile as the threshold for an abnormal NT thickness, resulting in a more sensitive and specific indicator for the detection of anomalous fetuses **(Forset JC et al 1997).** Therefore, it is mandatory to establish the normative distribution of fetal NT measurement. More recently, several other studies have been reported in Korean literature **(Biagiotti et al 1998 and Brady AF et al 1998)**, but they have relatively small sample size. The present study includes a very large number of pregnant women for analysis and thus offers normative data of the fetal NT thickness in a Korean population. Compared with the recent study by **Jou et al. (Ghezi F et al 2002**), which measured the NT thickness from 897 Taiwanese pregnancies between 9-14 gestational weeks, our results was similar to those. There is acontroversy as to the need to take the ethnic difference into account in the interpretation of NT measurements. Jou et al. suggested that, given the small but statistically significant differences, race-specific normative data should be used **(Ghezi F et al 2002**). Other authors concluded that it is acceptable to use a single standard, because screen positive rates in different groups are similar **(Bewley S et al 1995 and Ghi et al 2001).** In our study, the

mean NT thickness in normal Korean fetuses was 1.62 mm. Compared to the study in Taiwanese population by **Jou et al**. **(Ghezi F et al 2002**), mean NT thickness in our result was smaller than those by 0.1 mm. Otherwise, compared with the study in Caucasians by Thilaganathan et al. (**Bewley S et al 1995**), the difference was rather small (0.08 mm). Therefore, we suggest that the ethnic difference is not significant in interpretation of NT measurements. In our study, the incidence of NT thickness greater than or equal to 2.5 mm was 4.0%. This observation is in accordance with previous studies **(Aitken et al 1994** and **Caughy A et al 2002).** reported that the incidence of NT thickness greater than or equal to 2.5 mm in normal fetuses increased; 1.3% at CRL 30-39 mm to 13.2% at CRL 60-69 mm **(Caughy A et al 2002).** In our study, the incidence of NT thickness greater than or equal to 2.5 mm in normal fetuses was 2.2% at 11.0-11.9 weeks of gestation and increased to 12.5% at 14.0-14.9 weeks. Therefore, we confirm that a false positive rate increases with increasing gestational age, and each NT measurement should be examined according to the gestational age (weeks) and it must not be exceeds than 2.5 mm. Similar result was achieved by (**Szabo and Gellen** and **Kypros H et al**).

5.2 Conclusion:

- This study was proved its hypothesis that; the Nuchal Translucency is reliable and accurate in screening to diagnose fetal anomaly and trisomy 21 as early diagnosis of Down's syndrome and differentiates between normal fetus and abnormal fetus with trisomy 21 disorders of fetus.

- U/S scanning is a good diagnostic tool for screening and diagnosing the anomaly related to down syndrome and more accurate than lab investigation in showing dilatation of nuchal translucency NT measurements, (The accuracy of U/S in prediction of Down's syndrome is 79%, and the accuracy of lab investigation is 59.3%.

- In the excellent review of the subject of nuchal translucency screening in the first trimester by the role of biochemical screening. The most useful during the first trimester was found to be human chorionic gonadotropin (hCG) in the form of free Beta-hCG. The combination of free Beta-hCG, PAPP-A, and maternal age yields detection rates in the first trimester that are similar to those of second-trimester screening with 2 biochemical markers. The detection rates for trisomy 21 are approximately 60% together with maternal age at a 5% false-positive level.

- Fortuitously biochemical markers are largely independent of sonographic markers so that the combination of NT with first-trimester bio-chemical markers is more effective than either alone.

- Even mild degrees of increased NT may be significant when combined with biochemistry and maternal age, and this improves detection rates.

- Combining 2 serum markers (PAPP-Aand free Beta-hCG) with NT results in a detection rate of approximately 85%, for a 5% false-positive rate. Combining NT with biochemical markers also increases the detection rates for other aneuploidies.

- The U/S features of Nuchal Translucency (NT) that; the measurements of NT is more than 3 mm, it is means that there is sign of abnormality needs more investigations, The normal measurement is usually between 2-2.5mm, but there may be false positive sign.

- Measurements of nuchal translucency (NT)in ultrasound is usually are normal lays in the range between (1 to 2.5) if increased that means lays in abnormal range or dilated NT measurements . if the measurement is dilated or greater than 3 mm (are usually suggestive of Chromosomal abnormality). Other cases may be appears as large in measurements but after investigation fetus will be normal these lays in false positive results.

- There is group of women is relatively older and, hence, carries a higher risk of bearing a chromosomally abnormal fetus. It would be necessary to further evaluate if the reference range of NT thickness in naturally conceived singleton pregnancies could be employed in screening of Down's syndrome in multiple pregnancies conceived after assisted reproduction technology.

- By using discriminate analysis we found that the following Heart defects (VSD) is more recognizable in all outcomes of new baby's.

- None of these three tools (U/S scanning, freeBeta-hCG and PAPP-A) is accurate (100%) alone in differentiated between Chromosomal anomaly's positive or negative, but U/S scanning is very preferable in prediction of Fetal Chromosomal anomaly's as general because it can give the real measurements of Nuchal translucency. (Accuracy 79%), simple, cheep, save, and valuable.

- Free Beta-hCG ratio is more accurate than PAPP-A alone in differentiation between Fetal Chromosomal anomaly's.

- The Down syndrome positive range increases when the NT measurement increase from 3 mm and above and alpha –fetoprotein increase in the blood.

- The age is most risk factor of Down syndrome.
- In this study, it is found that, the ethnic difference is not significant in interpretation of NT measurements.

5-3 Recommendation:

- Pregnant women especially of age 20-45 years are advised to do U/S scanning routinely to exclude the presence of NT thickening.
- The author recommends that the Government should introduce the modern ultrasound machines and increase the training institutes of ultrasound for increasing the sonologists skills and experiences
- If there is any suspicion of Down's syndrome, fetus will be followed every month.
- The author recommended that the government should be increase the specialist hospitals for Obstetric
- The Biochemical serum screening is not usually available in Sudan, and it is expensive. According to its high values in diagnosis of Down's Syndrome, study advised the ministry of health and private laboratories to indorsing it, until it is becomes available to any patient.
- According to the high cost of scientific research which the researcher was faced, the government should appeal universities in Sudan and companies to support the researchers in order to improve plans of treating and management of such diseases.
- Further studies should be carried out in this field on many aspects such as increasing the number of patients, to show the relation between NT thickness and Biochemical markers include maternal serum AFP.

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