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Original Research Article

Is prenatal screening for Down syndrome needed in young pregnant women?

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ABSTRACT

Background: Down syndrome originally known as Mongoloid's idiocy is the most common autosomal disorder. Down syndrome (DS) can be detected by prenatal diagnosis which includes the triple marker screening test and chromosomal analysis.
Methods: The study population comprised of 100 pregnant females amongst the age group of 20-45 (32.10±4.86) years. Triple Marker Test was done followed by amniocentesis or CVS with karyotyping or FISH.
Results: Risk of <1:250 was considered high risk whereas ≥1:250 was considered as low risk. 32/45 (71%) were false-positive for Trisomy 21 detected as high risk by TMT. But there was good sensitivity and specificity for Trisomy 18.
Conclusions: It can be concluded that the triple marker test is indeed only a screening test for the DS and that it has to be confirmed with the help of chromosomal analysis. The higher maternal age is an important parameter in DS but nowadays, even ones with a lower maternal age can also have a child with DS. So, in general, now all women are recommended to go for biochemical screening during their pregnancy.

Keywords: Amniocentesis, Reproductive, Triple

INTRODUCTION

Down syndrome originally known as Mongoloid's idiocy is an autosomal disorder. Down Syndrome (DS) can be detected by two categories of prenatal tests i.e. screening tests and diagnostic tests. Prenatal screens estimate the chance of the foetus having DS. Most of these tests only provide a probability. Diagnostic tests can provide a definitive diagnosis with almost 100% accuracy.

Most screening tests involve a blood test and an ultrasound (sonogram). The blood tests measure quantities of various substances in the blood of the mother. Together with a woman's age, these are used to estimate her chance of having a child with DS. These blood tests are often performed in conjunction with a detailed sonogram to check for "markers" (characteristics that some researchers feel may have a significant association with DS).

New advanced prenatal screens are now able to detect chromosomal material from the foetus that is circulating in the maternal blood. Though these tests are not invasive, they provide a high accuracy rate. Still, all of these screens will not definitively diagnose DS. Prenatal screening and diagnostic tests are now routinely offered to women of all ages.

The diagnostic procedures available for prenatal diagnosis of DS are chorionic villus sampling (CVS) and

amniocentesis. These procedures, which carry up to 1% risk of causing a spontaneous abortion (miscarriage), are practically 100% accurate in diagnosing DS. CVS is usually performed in the first trimester between 9-11 weeks, whereas Amniocentesis is performed in the second trimester after 15 weeks of gestation.[1]

DS is usually identified at birth by the presence of certain physical traits: low muscle tone, a single deep crease across the palm of the hand, a slightly flattened facial profile and an upward slant to the eyes. Because these features may be present in babies without Down syndrome, a chromosomal analysis called a karyotype is done to confirm the diagnosis.

To obtain a karyotype, doctors draw a blood sample to examine the baby's cells. They use special tools to photograph the chromosomes and then group them by size, number, and shape. By examining the karyotype, DS can be diagnosed. Another genetic test called fluorescent in situ hybridization (FISH) can apply similar principles and confirm a diagnosis in a shorter amount of time.[2]

Aims and objectives

The present research aimed to study association of Down syndrome and Triple Marker test. The objective of this study is to verify the diagnostic accuracy of triple marker test in prenatal diagnosis and age risk of Down syndrome.

METHODS

The project was approved by Institutional Ethics Committee. Informed written consent was taken from the participants with compliance to PCPNDT Act 2003. Before going for prenatal diagnosis, a genetic counseling was done wherein the parent couple was detailed about the reason and significance of prenatal diagnosis, the risk factor of amniocentesis and CVS methods and the consequences of having a child with DS.

The study population comprised of 100 pregnant females amongst the age group of 20-45 years. Mean age group of the study subjects was 32.10±4.86 years. The subjects were categorized based on age into three groups i.e. <30, 30-35, and >35 years. Eighty-nine subjects could be followed up till the delivery, because of non-compliance of 11 subjects. Triple Marker Test was done followed by amniocentesis or CVS with karyotyping or FISH.

Chromosomal analysis revealed the karyotype of parent couple and fetus. Parent couple's karyotype additionally helped in counseling for future pregnancies, amniocentesis and CVS methods, the consequences of having a child with DS.

Triple marker test (TMT)

TMT is a screening test done on maternal blood which measures the amounts of three specific substances:

- α-Fetoprotein (AFP),
- β-hCG (Human Chorionic Gonadotropin) and
- Estradiol (µE3) in a pregnant woman's blood

The test is structured to find out the probability of the baby having certain chromosomal abnormalities like DS, Edwards syndrome and neural tube defects such as spina bifida or anencephaly.

Table 1: levels of triple markers in chromosomal abnormalities.

Chromosomal abnormality	AFP	hCG	µE3
Neural Tube Defects	⇧	-	-
Trisomy 21	⇩	⇧	⇩
Trisomy 18	⇩	⇩	⇩

If it shows a positive result, then one has to go for confirmatory diagnostic test which involves cytogenetic investigation. Normal values vary with gestational age. Corrections for maternal weight, diabetes mellitus, race, and other factors may be necessary. Screening can be done in the 1st trimester, 2nd trimester, or both (called sequential or integrated screening). Any of the 3 approaches are acceptable. Regardless of which is done, maternal levels of AFP should be measured during the 2nd trimester.[3] In the present study, the risk estimate obtained by TMT, was categorized into <1:250 and ≥1:250.

Amniocentesis

In amniocentesis, amniotic fluid is withdrawn for fetal cells for testing, including measurement of chemical markers (e.g. AFP, acetylcholinesterase). The safest time for amniocentesis is after 15-16-week of gestation. Amniocentesis has traditionally been offered to pregnant women >35 years because their risk of having an infant with DS or another chromosomal abnormality is increased. Occasionally, the amniotic fluid obtained is bloody.

Usually, the blood does not affect amniotic cell growth and is maternal; however, if the blood is fetal, it may falsely elevate amniotic fluid AFP level. Dark red or brown fluid indicates previous intra-amniotic bleeding and an increased risk of fetal loss. In Amniocentesis with experienced operators, risk of fetal loss is about 0.1-0.2%. Vaginal spotting or amniotic fluid leakage, usually self-limited, occurs in 1-2% of women tested.[4]

Chorionic villus sampling (CVS)

In CVS, chorionic villi are aspirated into a syringe and cultured. CVS provides the same information about fetal genetic and chromosomal status as amniocentesis and has same accuracy. However, CVS is done between 10-week of gestation and the end of the 1st trimester and thus provides earlier results. Therefore, if needed, pregnancy may be terminated earlier (and more safely and simply),

or if results are normal, parental anxiety may be relieved earlier. Depending on placental location (identified by ultrasonography), CVS can be done by passing a catheter through the cervix or by inserting a needle through the woman's abdominal wall. After CVS, Rh+(D) immunoglobulin 300 µg is given to Rh-negative un-sensitized women.

Errors in diagnosis due to maternal cell contamination are rare. Detection of certain chromosomal abnormalities (e.g. tetraploidy) may not reflect true fetal status but rather mosaicism confined to the placenta. Confined placental mosaicism is detected in about 1% of CVS specimens. Consultation with experts familiar with these abnormalities is advised. Rarely, subsequent amniocentesis is required to obtain additional information. Rate of fetal loss due to CVS is similar to that of amniocentesis (i.e. about 0.2%). CVS remains a viable alternative to amniocentesis for early prenatal diagnosis. Karyotyping and Amniotic cell cultures were performed.[5]

Karyotyping

Chromosome analysis is counting the number of chromosomes present in a specified number of cells (Metaphase spreads) followed by careful analysis of banding pattern of individual chromosome in selected cells.

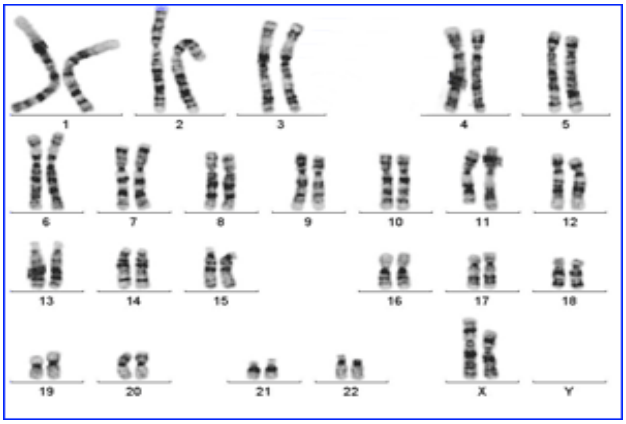

Figure 1: Normal female karyotype.

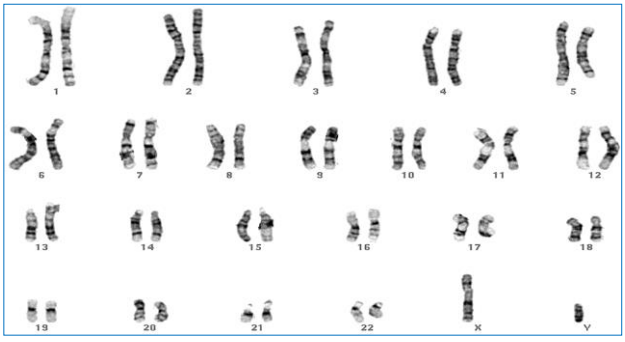

Figure 2: Normal Male karyotype.

The banding pattern of each chromosome is specific and can be shown in the form of a stylized ideal karyotype known as idiogram.[6]

The prenatal disorders are detected by comparing the patient's karyotype with the normal karyotype. With normal female and male karyotypes in Figure 1 and 2 respectively, DS which is a trisomy on chromosome 21 is shown in Figure 3.

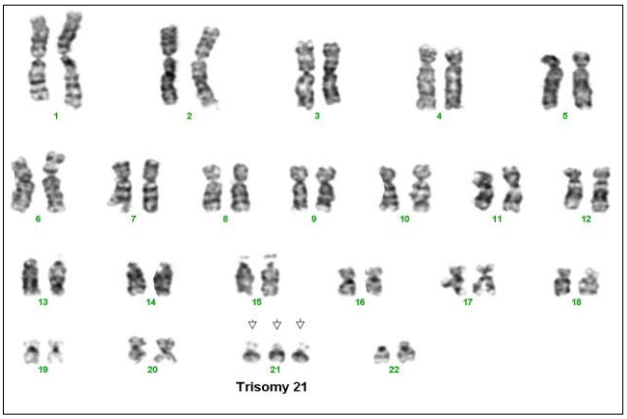

Figure 3: Down syndrome.

Statistical analysis

The data was expressed in terms of Mean±SD. Microsoft Office Excel 2016 and IBM SPSS Statistical Software was used to analyse the data. Appropriate statistical tests were applied and significance level of <0.05 was considered.

RESULTS

The response rate was 89%. Comparison amongst maternal age groups revealed statistically significant difference ($p<0.05$) in both high and low risk groups (Table 2).

As the age increases, the risk of having baby with DS or any other chromosomal abnormality increases. Table 3 and 3 depicts TMT results as per maternal age groups and risk groups respectively. Table 5-7 highlights outcome of confirmatory tests with respect to maternal age groups, risk groups and TMT results respectively.

Table 2: Comparison of maternal age groups w.r.t risk groups.

		Maternal Age (year)		Group	Total
		<30	30-35	>35	
Risk Group	<1:250	13	14	18	45
	≥1:250	13	23	8	44
Total		26	37	26	89

Pearson Chi-Square Test <0.05

Risk of <1:250 was considered high risk whereas ≥1:250 was considered as low risk. 32/45 (71%) were false-positive for Trisomy 21 detected as high risk by TMT. But there was good sensitivity and specificity for Trisomy 18. All 3 cases of Edward Syndrome were detected by TMT.

Table 3: TMT results as per the maternal age groups.

		Triple Marker Test Result			Total
		TMT -ve	TMT +ve for Tri 18	TMT +ve for Tri 21	
Maternal Age Group (year)	<30	11	1	14	26
	30-35	13	0	24	37
	>35	4	2	20	26
Total		28	3	58	89

Table 4: TMT results as per the risk groups.

		Triple marker test result			Total
		TM -ve	TM +ve for Tri 18	TM +ve for Tri 21	
Risk Group	<1:250	9	3	33	45
	≥1:250	19	0	25	44
Total		28	3	58	89

Table 5: Outcome of confirmatory tests w.r.t maternal age group.

		Outcome of confirmatory tests								
		46** with pericentric inversion on Ch 9	46**, 14ps+	46**, 15ps+	46**, 15ps+, Non-coding DNA in satellite region of Ch 15	Normal	Normal, Balanced translocation t (11;21) (p14:q11.2) in mother	Trisomy 18:47**, +18	Trisomy 21:47**, +21, 3 Signals for Ch 21	Trisomy 21:47**,+ 21
Maternal age Group	<30	0	0	0	1	22	0	1	2	2
	30-35	1	1	0	0	33	1	0	1	1
	>35	0	0	1	0	19	0	2	3	3

Table 6: Outcome of confirmatory tests w.r.t. Risk Groups.

		Outcome of confirmatory tests								
		46** with pericentric inversion on Ch 9	46**, 14ps+	46**, 15ps+	46**, 15ps+, Non-coding DNA in satellite region of Ch 15	Normal	Normal, Balanced translocation t (11;21) (p14:q11.2) in mother	Trisomy 18:47**, +18	Trisomy 21:47**, +21, 3 Signals for Ch 21	Trisomy 21:47**, +21
Risk Group	<1:250	0	1	1	1	32	0	3	1	6
	≥1:250	1	0	0	0	42	1	0	0	0

DISCUSSION

Down syndrome originally termed as mongoloid idiocy which is the most common autosomal disorder which was first reported in a man in 1866 named Langdon Down. Earlier literatures have proved that there is an increased risk for DS for maternal age over 30 or 35. This is due to errors in the chromosome division due to which there is a translocation and there is an extra chromosome on chromosome 21.[3,4] The triple marker tests can give false positive results also, so diagnostic tests are recommended more than triple marker test. History of DS has a recurrence chance of 1%, such couples have to take up the diagnostic tests.[5]

The characteristics of DS children were also studied. Chromosomal studies are important to understand the chromosomal aberrations like trisomies, translocations, deletions, duplications. These chromosomal aberrations lead to various kinds of disorders. With the help of the present study, it signifies that TMT is only for screening of DS. This test gives the probability of having a baby with chromosomal abnormality; hence, it has to be confirmed with the help of prenatal diagnosis- Amniocentesis and CVS. Research says that the maternal age is an important risk factor for Down syndrome. However, it has been noticed that the young mothers have also given birth to a DS baby. So now-a-days it is recommended that all women should go for biochemical analysis using Triple marker test during their pregnancy.[6,7]

Table 7: Outcome of confirmatory tests w.r.t TMT result.

		Outcome of confirmatory tests									
		46** with pericentric inversion on Ch 9	46**, 14ps+	46**, 15ps+	46**, 15ps+, Non-coding DNA in satellite region of Ch 15	Normal	Normal, Balanced translocation t (11;21) (p14:q11.2) in mother	Trisomy 18:47**, +18	Trisomy 21:47**, +21, 3 Signals for Ch 21	Trisomy 21:47**, +21	Total
TMT Result	TM -ve	1	0	1	1	25	0	0	0	0	28
	TM +ve for Tri 18	0	0	0	0	0	0	3	0	0	3
	TM +ve for Tri 21	0	1	0	0	49	1	0	1	6	58
Total		1	1	1	1	74	1	3	1	6	89

The advent of prenatal diagnosis has changed this traditional mode of diagnosis. The ability to detect an inborn error of metabolism, chromosome disorder, or congenital malformation in-utero early enough to allow termination of pregnancy has added a new dimension to genetic counseling. In place of confronting parents with the choice of planning further children in full knowledge of high risk of abnormality or limiting their families, it is now possible in a rapidly growing number of disorders to monitor risk pregnancies and ensure that children born will not be affected. In a sense this has added a positive element to the former inherent negativity of genetic counselling.[8]

In case the cytogenetic results of prenatal diagnosis show that the foetus is Down baby, the parents can continue the pregnancy or terminate the pregnancy. Such decisions are based on social and ethical considerations. If they do choose to continue with the pregnancy, they will need to understand that there is no cure for DS and the infant would benefit and survive with early medical assistance and developmental interventions during the early years. The following actions we believe that if they take will help them raise the child: Assemble a team of professionals – The parents will be put in touch with expert professionals dealing with physical therapy, speech therapy and occupational therapy.

Seek out other families – They could also find support and speak to people who have raised kids suffering from Down syndrome. They could go to their special societies or schools and find out in person. If the ethical problems with termination of pregnancy are present, they are usually in the technology of prenatal diagnostics as: the safety of amniocentesis, the problem of growing amniotic fluid cells, the use of amniotic fluid itself, the number of disorders which may be detected in-utero in the early stages of gestation, the certainty of diagnosis, and the extension of the list of disorders capable of diagnosis. However, until science has the knowledge to treat some of the more serious, sometimes fatal genetic disorders, the best option is prevention by resorting to prenatal diagnosis and genetic counselling.[9]

According to Pre-Conceptional Prenatal Diagnostic Techniques (PCPNDT) Prohibition of Sex Selection Regulation and Prevention of Misuse Act (2003) in prenatal diagnostic cases, sex is not disclosed to the patient. Whenever the patient comes for the prenatal diagnosis, their consent regarding non-disclosure of sex is taken. However, with the widespread availability and improved safety of amniocentesis, the American college of obstetricians and gynecologists recommends all pregnant women be offered amniocentesis to assess the presence of fetal chromosome disorders.

CONCLUSION

It is recommended that all women should go for biochemical analysis using Triple marker test during their pregnancy.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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