

Trisomy 21 Testing in Second Trimester: Reliability Comparison of Triple and Quadruple Testing in Pakistan

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ABSTRACT

Objectives: Down syndrome is a disorder that affects one in every 700 babies born, and it is one of the most frequent causes of developmental delay (DS). In Pakistan, prenatal screening for Down syndrome is not being practised according to any approved protocol. The biochemical screening that takes place during the second trimester is still carried out using the triple test. Although the quadruple test has a better sensitivity and specificity than other diagnostic methods, its usage in routine medical settings is not advised. The purpose of this study was to explore the second trimester screening for trisomy 21 (quadruple test with genetic sonogram) in order to determine its sensitivity and accuracy in comparison to biochemical testing.

Place of Study: District Headquarters Hospital, Gujranwala

Duration of Study: January 2021 to December 2021

Methods: This retrospective observational study was carried out in a Fetal Medicine Centre with the purpose of analysing the odds of being affected with Down syndrome, given a positive risk (OAPR) upon screening in the quadruple test; triple test and quadruple test plus a genetic sonogram for highrisk singleton pregnancies (in view of advanced maternal age; an anomaly scan showing some abnormality, etc.). Given the presence of a positive risk factor, the goal of this study was to ascertain the likelihood of having a child impacted by Down syndrome.

Practical Implication: To explore the second trimester screening for trisomy 21 (quadruple test with genetic sonogram) in order to determine its sensitivity and accuracy in comparison to biochemical testing.

Results: The discovery of trisomies was made after an investigation into 3,042 highrisk pregnancies with a singleton baby. 327 pregnant women who have received positive findings from a triple test, quadruple test, or quadruple test in addition to a genetic ultrasonography opt to have an amniocentesis. This decision was made after the ladies learned that their unborn child may have a genetic disorder. It was discovered that 20 of the developing babies were affected by Down syndrome. The OAPR for the quadruple test was demonstrated to be significantly higher when compared to the OAPR for the triple test (1:29.1 as compared to 1:39.2). It was found that the combination of the triple test and the genetic sonogram had the highest OAPR of 1:7.

Conclusions: The triple test combined with a genetic sonogram is the most effective tool for screening for trisomy 21, and it has the potential to cut down on the number of unnecessary amniocentesis operations carried out in high-risk populations.

Keywords: Down syndrome, genetic, quadruples test, second trimester screening, triple test, ultrasound

INTRODUCTION

The most frequent reason for developmental delay is Down syndrome (DS), which is also responsible for 16-30% of those who have intellectual difficulties.

According to several studies that have been made public and the research that has been made public, the birth prevalence of Down syndrome in Pakistan ranges from one in 900 to one in 1100. This information comes from the research that has been made public. According to the findings that have been made public, around one in every three hundred infants born each year in Pakistan are diagnosed with Down syndrome. Even though more information regarding screening for Down syndrome is becoming available in both the public and private sectors, there is still no comprehensive methodology that can be accessed either from the government or from professional organisations¹. This is the case despite the fact that more information regarding screening for Down syndrome is becoming available. The only screening test that can detect aneuploidies in people who are pregnant in Pakistan is a genetic ultrasonography, which is performed between 18 and 20 weeks of pregnancy on average. This is the scenario in a great deal of the country's regions that have restricted access to the resources that are available. If it is carried out in a standard lowvolume clinic, this genetic ultrasonography has a detection rate (DR) of 48 percent and a false positive rate of three percent. On the other hand, if it is carried out in clinics that are specifically dedicated to obstetrics and gynaecology, it has a detection rate (DR) of 75%. Even in this day and age, it is common practise in many parts of the country to screen for foetal aneuploidies in the second trimester of pregnancy using maternal serum in the form of the triple test [serum alpha foeto protein, serum total beta human chorionic gonadotrophin (HCG), and serum unconjugated estriol]². This screening is performed to determine whether or not the foetus

has an abnormal number of copies of its genetic material. In spite of the fact that the quadruple test (which includes an additional biochemical marker known as serum inhibin A in addition to the triple test) is known to have a higher sensitivity in comparison to the triple test, it is still not being used for screening of aneuploidies on a widespread scale in Pakistan as a result of a lack of awareness, financial constraints, and the availability of laboratories. In the United States, the quadruple test is utilised for screening of aneuploidies on the combination screening performed during the first trimester of pregnancy is the primary method that is utilised when screening individuals for Down syndrome. The free blood beta-HCG level, the presence of pregnancy-associated plasma protein (PAPP-A) in the serum, and an ultrasonography of the nuchal translucency and nasal bone (NT/NB) are the three components that make up this test³. This test has a sensitivity of 90.7 percent while at the same time having a specificity of 91.4 percent. The percentage of false positives is 1.4%, according to the findings. The objective of this study was to compare the odds of being affected with Down syndrome (i.e. diagnosing trisomy21) given a positive risk of the quadruple test to the odds of being affected given a positive risk (OAPR) of the triple test. The quadruple test was used because it has a higher likelihood of detecting Down syndrome than the triple test does. In addition, the purpose of the study was to examine the improvement in DRs that can be achieved by the triple test when it is paired with a genetic sonogram between the ages of 18 and 20 weeks of gestation in a tertiary care environment^{4, 8}.

MATERIAL & METHODS

Having a pregnancy with Down syndrome is something that can be confirmed with chorionic villous sampling (CVS) or amniocentesis. If a woman's screen risk is high, then there is a greater possibility

that she will have a child with the condition. This possibility is taken into account by OAPR. When a screening test has a high overall pregnancy loss prevention rate, also known as an OAPR, more affected pregnancies will be successfully found for every miscarriage that is generated by intrusive testing. In other words, the screening test will outperform the invasive test.

To minimise the number of women who are offered invasive procedures, which will, in turn, reduce the number of women who miscarry healthy foetuses, it is vital that the falsepositive rate (FPR) or the screen positive rate be kept as low as is practically practicable. The overall accurate positive rate (OAPR) for this inquiry was calculated by dividing the total number of genuine positive results by the total number of false positive results.

All of the women who had a positive screen result for either the triple test or the quadruple test were given the option of either undergoing a risk reassessment scan (in which case, new risks would be generated after performing a genetic sonogram in conjunction with the quadruple test) or proceeding directly with diagnostic testing using amniocentesis, fluorescent in situ hybridization, and karyotyping. In the case of the former, new risks would be generated after performing Amniocentesis and further testing were performed on the amniotic fluid that had been aspirated using an 18gauge needle and guided by ultrasonography. The total amount of amniotic fluid that was tested was 30 millilitres. The ultrasonic tests were performed with real-time, high resolution scanning using a convex probe with a frequency of 3.5 MHz, a volume angle of 5–95 degrees, a frame rate of 395 hertz, and a depth of 3–25 centimetres. All of these parameters were measured at the same time.

RESULT

The duration of the study was eight years, and throughout that time there were a combined total of 3,175 high-risk singleton pregnancies that were analysed. The demographic features of the population that was the focus of the inquiry are presented. Because the triple test was not carried out at the study site, a total of 1402 highrisk pregnant women carrying only one kid were required to undergo the examination at a separate location. These individuals had previously been to another facility where they had undergone a triple test. They were referred to us as a result of either a screen positive triple test, an anomaly scan in light of any questionable finding, or because they had missed their first trimester combination screening. After undergoing testing for the triple test, 327 of these 1402 individuals had results that indicated they were positive for it. All of these patients, numbering 327 in total, who had a positive screening were then given indepth counselling and were given the option to either undergo risk reassessment (a triple test in addition to a genetic ultrasonography for soft indicators), NIPS (beginning in the year 2015), or amniocentesis. NIPS is a noninvasive prenatal screening test. Among the 208 patients who elected to have an amniocentesis performed immediately, there were determined to be 6 patients who were affected by trisomy 21. 121 pregnant women made the choice to undergo risk reassessment. During the risk reassessment process, it was discovered that 11 patients had a positive screening result; as a consequence, these 11 patients had amniocentesis. Trisomy 21, a genetic disorder, was identified in two of these 11 patients as the underlying cause of their illness.

Following further examination, none of the remaining 110 patients who had been subjected to risk reassessment and had been determined to have a negative screening result were discovered to have trisomy 21. During the subsequent followup, the problem was identified in two out of 1161 patients who had previously been screened and found to be negative for trisomy 21 using all three tests. A total of 1640 pregnant women who were carrying a highrisk singleton child were put through a quadruple screening during the course of the experiment. When these 1640 patients were given the triple test, there were 308 of them who had screening findings that were positive. Those women who had a positive screening quadruple test were also provided with indepth

counselling, and they were given the option of having their risk reevaluated (which consisted of a quadruple test in addition to a genetic ultrasonography for soft markers). 155 women, or 49.8% of all women, made the decision to have an amniocentesis performed immediately, and six of those patients were identified with trisomy 21. There were 156 women who made the conscious decision to have a reevaluation of their risk performed on them by submitting themselves to a triple test in addition to a genetic sonogram for soft indicators. During the screening phase, twenty of these 156 women tested positive for trisomy 21, and three of the patients who had amniocentesis were ultimately identified with the disorder.

When the remaining 136 patients who had a negative screening using risk re-assessment were followed up, it was discovered that one of those patients had trisomy 21.

During the initial screening, there was only one patient out of 1374 who tested negative for having triple chromosomes. However, during the subsequent testing, there was one patient who tested positive for having trisomy 21. Women who were screened positive either on triple test or quadruple test or quadruple test with soft markers directly elected for amniocentesis in our study during that period, with none of the patients getting NIPS. This was done during the time period. This was owing to the expensive expense, poor data on the sensitivity of NIPS initially, increased concern of patients due to screen positive biochemical tests, and the dread of getting intrusive testing done if NIPS comes back positive. All of these factors contributed to this conclusion. There were a total of 3 175 highrisk pregnancies that were examined, and out of those, 21 foetuses were found to have trisomy 21. As a consequence, the incidence of individuals at our centre who have trisomy 21 is one in every 151. It was found that the OAPR for the triple test was 1:39.2 which extremely low value is.

Demographic character	Triple test (n=1402)	Quadruple test (n=1640)
Mean age (yr)	35.9	34.8
Mean weight (kg)	83.9	86.7
Mean BMI (kg/m ²)	21.7	22.9
Mean gestational age at sampling (months of gestation)	17.8	18.1
BMI, body mass index		

Test	Total positive	True positive	OAPR
Triple test	327	7	1:39.2
Quadruple test	308	9	1:29.1
Quadruple plus soft markers	30	4	1:7
OAPR, odds of being affected given a positive risk			

DISCUSSION

According to the findings of recent investigations, the odds of an individual getting Down syndrome are approximately one in 13613. The most prevalent inherited cause of developmental delay can affect as many as one child in every 6924 live births and has an incidence of up to one in every 6924 live births. The likelihood of an individual being born with Down syndrome (DS) climbs steadily up until the age of 32 years, at which point it begins an exponential ascent that continues up until the age of 45 years, after which it reaches a plateau¹¹. The risk of having a child with DS remains constant after this age. After this age, there is no longer an increased chance of getting Down syndrome. According to the findings of the National Down's Syndrome Cytogenetic Register in the United Kingdom (UK), if screening methods hadn't been improved between the years 1989 and 2008, the ongoing increase in the average age of mothers would have resulted in a 48% increase in the number of live births involving individuals with Down's syndrome. This would have been the case despite the fact

that the number of live births involving individuals with Down's syndrome has decreased overall since 1989. This would have been the result of an increase in the number of people with Down syndrome who gave birth to healthy children. In addition to the age of the mother, biochemical and ultrasonographic markers that have been created since the early 1980s have significantly contributed to an increase in the sensitivity of screening programmes.^{15, 16} The most cutting-edge screening technology that is now accessible does not call for the participant, who is being screened, to undergo any form of intrusive procedure. This approach is the next step forward in the field of sequencing technology. In the event that it is determined that a pregnancy has a positive test for DS on biochemical screening, whether on a dual test or a triple/quadruple test, the alternatives that are available include combining the dual test with an NT/NB scan that is performed by a specialist in foetal medicine. In addition, in the event that it is determined that a pregnancy has a positive test for DS on biochemical screening, the alternatives that DS on the screening of biochemicals. This is one of the options that are open to you at this time^{11, 13}. The diagnostic reliability of the combination screening that was performed during the first trimester is increased to between 93 and 95 percent as a result of this, while the false positive rate is maintained at 3 percent. A pregnancy that has a positive screening result for Down syndrome on a quadruple test and a risk reassessment (in which the quadruple test is paired with the genetic ultrasonography) offers a DR of 80% with an FPR of 3%. This is because the FPR is calculated by dividing the DR by the number of times the test was positive. A pregnancy that has a positive screening result for Down syndrome on a triple test and a risk reassessment (in which the triple test is matched with the genetic test) is considered to have an increased risk of having a child with Down syndrome. In spite of this, the CVS and the amniocentesis tests continue to be the tests of choice for diagnostic reasons when it comes to detecting whether or not an individual has Down syndrome. It is generally agreed upon that the invasive nature of these tests brings with them a risk of iatrogenic foetal loss that ranges between 0.7 and 1%. Numerous developing nations, Pakistan included, have not yet integrated an adequate screening strategy into their national prenatal screening programmes. This is one of the major challenges facing the field. On the other hand, in the Western world, screening can begin as early as the first trimester, which is when the initial ultrasounds are performed. This is in contrast to the Eastern world, where screening cannot begin until the second trimester. During the screening process that is performed for each trimester, both the dual test and the NT/NB scan are utilised in conjunction with one another. At the beginning of the second trimester, which is 16 weeks into the screening procedure, a triple test is performed. This marks the beginning of the second trimester. Two weeks after that, at the 18-week milestone, a genetic sonogram is performed to estimate the likelihood of having a child with Down syndrome.

The following is a list of the findings from the genetic analysis:

Once more, the results of the ultrasonography and the triple test, in addition to the results of the screening that was performed during the first trimester, are combined. The sequential screening for developmental delay is a method that has a DR of 95% and ought to be utilised in each and every location of the world. It is really necessary for each and every one of us to make use of this strategy¹⁰.

In Pakistan, there are a significant number of organisations that are classified as belonging to both the public and the private sectors. The screening process typically starts in a private setting in the second trimester of pregnancy. However, the triple test, which has been around since 1988 and was initially developed as a biochemical screening tool for Down syndrome in the first trimester, is still increasingly used in that stage of pregnancy⁷. In

the Pakistani circumstance, the prolonged combination test for the first trimester, which includes a DR, has not yet reached the point where it is considered to be standard practise. It is well known that the reliability of the quadruple test in the screening process for Down syndrome is noticeably higher than that of the triple test. The SURUSS examination, which was carried out in 2003, produced findings that were comparable; it discovered a DR of 77% for triple testing and a DR of 84% for quad testing while maintaining the FPR at 5%. It was found that the quadruple test is a more effective screening tool; however, it could not be used in national screening protocols because the only commercially available assay for inhibin^{5,11} A was not suitable for use in a routine laboratory (insufficiently stable, and the intra batch assay variation was excessive, 17%). Because of this, the quadruple test could not be used in national screening protocols.

CONCLUSION

The triple test combined with a genetic sonogram is the most effective tool for screening for trisomy 21, and it has the potential to cut down on the number of unnecessary amniocentesis operations carried out in high-risk populations.

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