Original Article
Cent Asian J Med Sci. 2018 Sep;4(3):230-237. https://doi.org/10.24079/CAJMS.2018.09.009

Central Asian Journal of Medical Sciences
CAJMS

# The Use of Double Test Biomarkers to Predict Fetal Nuchal Translucency Thickness in the Diagnosis of Trisomy 21: A Compromise between Sensitivity and Specificity

**Ganzug Jugder[1], Oyunchimeg Unshigbayar[2], Purevdorj Ichinkhorloo[1], Tsolmon Boldoo[3], Erkhembulgan Purevdorj[4]**

[1]Department of Molecular Biology and Genetics, School of Biomedicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia; [2]The Laboratory of Medical Genetics, National Center for Maternal and Child Health, Ulaanbaatar, Mongolia; [3]Research Department, National Center for Communicable Diseases, Ulaanbaatar, Mongolia; [4]The Institute of Medical Sciences, Ulaanbaatar, Mongolia

**Submitted:** 12 August, 2018
**Revised:** 16 September, 2018
**Accepted:** 20 September, 2018

**Corresponding Author**
Ganzug Jugder, MSc
Department of Molecular Biology and Genetics, School of Biomedicine, Mongolian National University of Medical Sciences, Ulaanbaatar 14210, Mongolia
**Tel:** +1-719-325-9516
**E-mail:** ganzugjugder@gmail.com



**Objectives:** The aim of study is to determine the sensitivity and specificity of screening for fetal trisomy 21 in the first trimester of pregnancy using a combination of maternal serum biomarkers and ultrasonography. The objectives of this study were to first duplicate and confirm within the Mongolian population, the results of other similar studies. Our results corroborated closely with those of other studies and further allowed us to draw conclusions and recommendations unique to Mongolia. A second objective was to apply these results to everyday obstetric practices within Mongolia and advance the state of prenatal care for all Mongolian women. **Methods:** Obstetric History: The obstetric history was recorded, and serum biomarkers were measured in 415 pregnancies.The degree of risk for trisomy 21 and other fetal aneuploidy were calculated using maternal age, the results of maternal serum pregnancy-associated plasma protein-A, free beta human chorionic gonadotropin, and fetal nuchal translucency thickness. Grouping of Test Subjects: Using a cut-off risk estimate of 1 in 300, the women were grouped into positive and negative screening groups. Sensitivity and specificity of the screening method were calculated from the available data. **Results:** Sensitivity of the screening test for fetal trisomy 21 was 66.6% and for all fetal aneuploidy was 83%. Specificity of the screening test for fetal trisomy 21 was 87.5% and for all fetal aneuploidy was 97.8% and the false positive rate was 2.2%. Our results are consistent with the results obtained in other international studies. **Conclusions:** Our study is unique in that it is the first such scientific examination of mothers at risk for congenital abnormalities in Mongolia. This study provides empirical evidence that the combination of the double test, maternal age, and fetal nuchal translucency thickness is an effective prenatal screening method for fetal aneuploidy pregnancy outcomes in Mongolia.

**Keywords:** Down Syndrome, First Trimester Serum Biomarker, Fetal Nuchaltranslucency Thickness, Sensitivity, Specificity.

## Introduction

Screening is an effective for detecting Down syndrome (DS) in pregnancies identified at high risk for fetal aneuploidies. First-trimester screening, using fetal nuchal translucency thickness (NTT) combined with maternal age and the serum markers pregnancy-associated plasma protein-A (PAPP-A) and free betta human chorionic gonadotropin (βhCG), have been demonstrated in several large studies to have comparable or greater accuracy than other methods [1, 2].

In trisomy 21 during the first trimester of pregnancy, the maternal serum concentration of PAPP-A is decreased while free βhCG is increased [3, 4]. In trisomy 13 and 18 maternal serum concentration of PAPP-A and free βhCG are both decreased [5].

Studies have shown that NTT measurements between 11and 14-weeks' gestation, when combined with maternal age, yielda detection rate (DR) of 75%, with a false positive rate (FPR) of just 5% [6]. When these two tests are combined with PAPP-A and free βhCG, the DR of chromosomal abnormalities can increase up to 85-90% with a FPR of 5% [7].

Various international experimental efforts have endeavored to advance DS screening and both DR and FPR using this combined approach [8, 9]. In the Netherlands, DS screening currentlyhas a DR of 75.9% and an FPR of 3.3% [10].

Therefore, it is reasonable to conclude that using this combined testing method, more accurate test that provides a high DR and a relatively low FPR is achievable. The primary advantage of the combined test is the availability of the results within the first trimester, enabling karyotyping via chorionic villus sampling (CVS) and amniotic fluid cells (AFC) and early surgical termination of the pregnancy, if indicated [11].

The objective of study was to determine the sensitivity and specificity of screening for risk for trisomy 21 and other fetal aneuploidy in the first trimester of pregnancy by a combination of maternal serum biomarkers and ultrasonography in Mongolian population.

## Material and Methods

The obstetric history was recorded and serum biomarkers were measured in 415 pregnant women receiving obstetric care at the National Center for Maternal and Child Health Laboratory and Genetic Counseling Cabinet in Ulaanbaatar, Mongolia. Data were collected during visits at 11 to 13 weeks of gestation. For all 415 pregnancies, risk was calculated based on maternal age, the results of maternal serum pregnancy, PAPP-A, βhCG and fetal NTT. Screening, using the combined markers, then revealed those women at increased risk of carrying a fetus with risk for trisomy 21 and other fetal aneuploidy.

### Laboratory Measurements

#### Specimen collection and preparation

For maternal serum, standard universal precautions for venipuncture were observed. The specimens were stored at 2-8$^0$ C for up to 24 hours and the samples kept frozen at -70$^0$ C. Repeated freeze-thaw cycles of samples was avoided. All reagents and samples were brought to room temperature (18-25$^0$ C) before use.

#### Laboratory Serum Blood Tests

These bio-chemical markers PAPP and βhCG analyzed with enzyme-linked immunosorbent assay (ELISA) using kits from Dynex Technologies 2®. DRG International, Inc. The microtiter wells (kits) are coated with a polyclonal anti PAPP-A and βhCG antibody. An aliquot of the patient sample containing PAPP-A and βhCG was incubated in the coated well with a sample buffer. After incubation a kit complex was formed with anti-PAPP-A and βhCG antibody peroxidase conjugated. Having added the substrate solution, the intensity of color developed was visually proportional to the concentration of PAPP-A and βhCG in the serum sample.

#### Ultrasonography

Ultrasonography was utilized to assess fetal NTT at 11 to 13 weeks'gestation and was performed by a Fetal Medicine Foundation (FMF) trained ultra-sonographer adhering to standardized protocols.

#### Measurement parameters sensitivity and specificity

Sensitivity refers to the ability of a test to correctly detect the number of individuals with abnormalities out of all individuals within the test group and is expressed as a percentage. Sensitivity is also referred as detection rate (DR) or true positive rate (TPR), again as a percentage. The complement of sensitivity is the false negative rate (FNR) [12, 13].

Specificity refers to the ability of a test to correctly detect

the proportion of individuals without abnormalities out of all individuals within the test group. It is expressed as a percentage. Specificity is also referred as the true negative rate (TNR) as a percentage. The complement of specificity is the false positive rate [12, 13].

The lower limit of detection (LLD), which is a measure of test sensitivity, was determined by assaying replicates of the zero and standard curves. The mean signal of zero +2 standard deviations in the amount of the substancefrom the standard curve is the LLD. This value represents the smallest amount of a substance that can be distinguished from the absence of the substance with 95% confidence.

### Statistical analysis

The descriptive statistics for continuous variables were expressed in mean ± standard deviation or median (minimum-maximum), while nominal variables were expressed in the number and percentage (%). The significance of the difference between the mean values of the groups was evaluated using the Student's t-test.

The average serum biomarker was estimated at a 95% confidence interval (CI). After the difference of the variables was normalized, the dissimilarity between the variances was calculated using Pearson's quadratic variables to determine the difference between the variables and the statistically significant difference was determined when the mean difference between the group was less than $p<0.05$.

Noninvasive prenatal test sensitivity, specificity, positive value and negative predictive value were calculated for DS.

The individual risk of each pregnancy was calculated following ultrasound by using FMF software that takes the biomarker values of maternal age, and fetal NTT into account. Using a cutoff value of 1 in 300, all the participants were grouped into either screen-negative (if the risk was < 1 in 300) or screen positive (if the risk was ≥ 1 in 300). The fetal chromosomal status of the screened positive participants was confirmed by CVS.

The sensitivity & specificity analysis was done using Clinical Decision Making Program software from the Department of Family Medicine, University of Oklahoma Health Sciences Centerand Microsoft Excel, SPSS 20, and STATA 14.2 software.

### Ethical statements

The study protocol was approved by the Ethical Committee of the Biomedical Department of the Ministry of Health, Mongolia.

## Results

### General characteristics of the study population

Of the 415 participants within the study group, 5 were diagnosed with twins. The mean maternal age was 32.4±6.20 (range of 20-47 years), while 174 participants were over 35 years of age (41.9%). The mean maternal body weight, in kilograms, was 65.2±10.1 (range of 44-120 kg). All study participants had at least one previous pregnancy. Of these, 97 pregnancies resulted in miscarriages (23.4%), 66 women experienced in utero fetal demise (16.0%), there were 38 premature births (9.1%), and 28 stillbirths (6.7%). These events were the motivating consideration for 229 (55.1%) women to seek professional obstetric care during their subsequent pregnancy. Thirty-five women (8.4%) smoked during the pregnancy, four (1.0%) were occasional smokers, and three hundred seventy-seven (90.6%) were non-smokers. One hundred eighty-eight (45.3%) were exposed to second-hand smoke. The patient characteristics expressed as mean values with standard deviation or range are listed in Table 1.

**Table 1.** Obstetric and smoking history of the study participants.

| Parameter | Value |
|---|---|
| Total | n=415 |
| Maternal characteristics | |
| Age (years) | 32.4 (20-47) |
| Body weight (kg) | 67.2 (44-120) |
| Obstetrics characteristics | |
| Primary | 138 (41.2%) |
| Repeated | 277 (58.8) |
| Twin pregnancy | 5 (1.2%) |
| Termination of pregnancy ( by repeated numbers) | |
| Premature birth | 38 (9.1%) |
| Fetal demise | 66 (16.0%) |
| Miscarriage | 97 (23.4%) |
| Ectopic pregnancy | 14 (3.4%) |
| Stillbirth | 28 (6.7%) |
| Elective abortion | 159 (38.4%) |
| Smoking status | |
| Smoker | 35 (8.4%) |
| Occasional smoker | 4 (1.0%) |
| Non-smoker | 377 (90.6%) |
| Second-hand smoke exposure | 188 (45.3%) |

Elevated maternal serum biomarkers values in pregnancies and fetal NTT

During the first trimester for all 415 pregnancies, the mean serum biomarkers of the screen-negative group were PAPP-A 11.5±5.2 ng/ml ($p<0.001$), βhCG 36.8 ± 21.6 mIU/ml ($p<0.05$), and NTT 1.9±0.7 ($p<0.001$).

Of the 415 pregnancies studied, 403 (97.1%) screened negative while 12 (2.9%) screened positive.

The biomarkers from the group that screened negative were expressed as the median with (95%) confidence intervals (CI) of (CI 4.7-30.1) PAPP-A, (CI 10.1-88.1) βhCG, and (CI 1.7-2.0) fetal NTT in the first trimester and are shown in Table 2.

The 12 cases that screened positive for aneuploidies and congenital defects using PAPP-A and βhCG both had below normal values. The mean serum biomarkers of the group that screened positivein the first trimester were PAPP-A 6.6 ±3.3 ng/ml ($p<0.002$), βhCG 28.9 ±18.1 mIU/ml, and fetal NTT 2.6 ±1.2 mm ($p<0.001$). The statistical mean biomarker values of gestational ages and fetal NTT values with standard deviation and mean are shown in Table 2.

In the 12 positive samples, the NTT values was normal in 7 cases while these values increased in the other 5. First trimester biomarker NTT values alone were DR 50.0% (21.1-78.9) and FPR 6.7% (4.5-9.6) of Fetal NTT screened.

In the positive-risk group, those with a maternal age under 24 years had a NTT of 3.8 mm ($p<0.002$), while the 30-34-year age group (one participant) NTT measured 4.4 mm and those over 40 years of age had NTT measurements of 3.4 mm ($p<0.001$). The fetuses in the 25-29-year-old group were the exception and normal NTT measurements. This indicates that the women in three of these age groups were at increased risk for an abnormal fetus due to above normal NTT measurements (greater than 1.6 mm to 1.9 mm).

The biomarker detection rates were 83.3% (CI 51.6-97.9) for PAPP-A, 91.7% (CI 61.5-99.8) for βhCG, with a 2.5% (CI 1.2-4.5) FPR. Figure 1 (A) and (B) illustrates these values, showing the fetal chromosomal and congenital defects in both screen-negative and screen-positive pregnancies.

Calculation of the sensitivity & specificity of the combined screening test for fetal aneuploidies

During the study, two pregnancies miscarried, and both fetuses were lost. One of these two screened negative while the other was screened positive. The patient who screened negative miscarried at 12 weeks while the one who screened positive miscarried at 11 weeks. Both patients tested positive for trisomy 13 and trisomy 21 using cytogenetic analysis as shown in Table 3 as shown below.

The method for calculating the sensitivity for trisomy 13 and trisomy 21 and its result is:

The method for calculating the specificity of the combined screening test for fetal aneuploidies and its result is:

FPR of the combinedscreening test for fetal aneuploidy was calculated as follows:

100% -specificity = 100% -97.8% = 2.2 % FPR.

The model-estimated detection and false-positive rates, using a 1 in 300 term cutoff, together with the prospectively observed rates shows a considerable reduction in the FPR along with a small increase in the detection rate.

**Table 2.** Comparison of gestational age (in weeks) with the serum biomarker PAPP-A, βhCG values and NTT between those that screened negative and screened positive.

| Weeks | N | Negative group | | | N | Positive group | | |
|---|---|---|---|---|---|---|---|---|
| | | PAPP-A | βhCG | NTT | | PAPP-A | βhCG | NTT |
| 11 | 81 | 8.8 ± 3.9 | 35.9 ± 20.8 | 1.8 ± 0.6 | 2 | 3.3 | 41.2 ± 0.8 | 1.6 ±0.3 |
| CI | | (4.7-16.1) | (10.1-86.4) | 1.7-2.0) | | 3.2-32.2) | (34.4-48.0) | (-0.9-4.1) |
| 12 | 158 | 10.0 ± 4.0 | 37.1 ± 21.6 | 1.8 ± 0.8 | 6 | 6.7 ± 3.2 | 22.7 ± 8.2 | 2.6 ± 1.2 |
| CI | | (4.9-23.1) | (8.7-85.8) | (1.7-2.0) | | (3.3-10.1) | (14.2-31.2) | (1.3-3.8) |
| 13 | 164 | 13.2 ± 5.9 | 36.8 ± 22.1 | 1.9 ± 0.7 | 4 | 8.1 ± 3.6 | 31.9 ± 30.1 | 3.3 ± 1.1 |
| CI | | (7.0-30.1) | (10.2-88.1) | (1.8-2.0) | | (2.3-13.8) | (-16.0-79.8) | (1.5-5.0) |
| Total | 403 | 11.1 ± 5.2 | 36.8 ± 21.6 | 1.9±0.7 | 12 | 6.6 ± 3.3 | 28.9 ± 18.1 | 2.6 ± 1.2 |
| CI | | (8.0-11.6) | (32.1-38.9) | (1.8-1.9) | | (4.5-8.7) | (17.4-40.4) | (1.9-3.4) |

The data expressed as a mean with 95% confidence intervals.

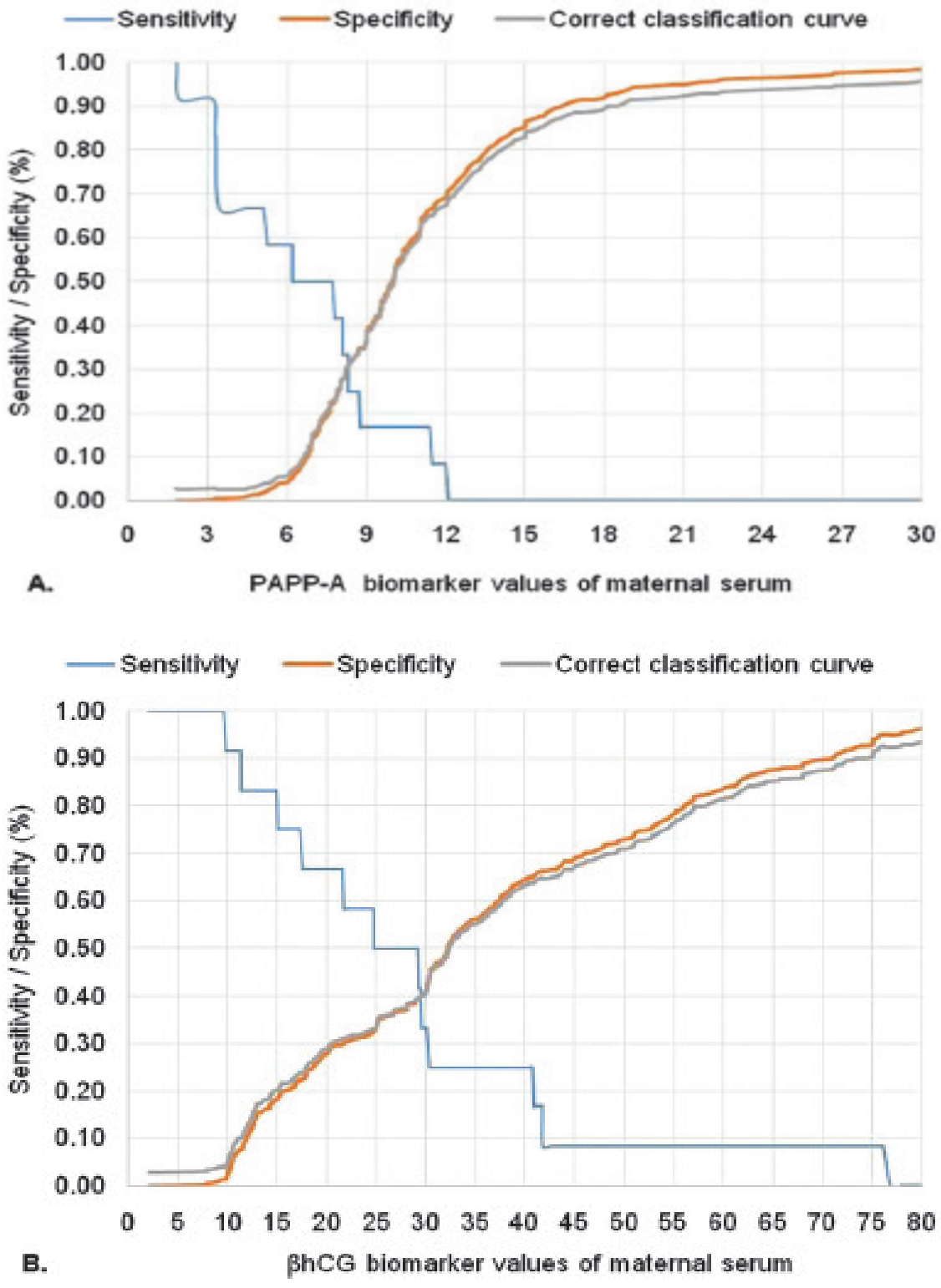


**Figure 1.** Calculations of sensitivity, specificity of screening of double test for all pregnancies and the correct classification curve for fetal abnormalities.

(A) Sensitivity and specificity of PAPP-A.

(B) Sensitivity and specificity of βhCG.

## Discussion

Antenatal screening and prenatal diagnosis for DS and other congenital defects was first performed in the 1979's using the limited methods of advanced age or previous history of aneuploidy. In the 1980's the association of DS with abnormal levels of certain serum markers was discovered, and maternal serum screening was developed which further improved the detection rate [14-17].

DS, one of the most common fetal aneuploidies, is associated with mental retardation and a range of lifelong physical disabilities in the affected individual. Our intent was to determine the sensitivity and specificity of a screening method using the combination of maternal age and NTT for all fetal aneuploidy including DS. While there are other, similar, studies in the literature, none address the distinctive population Mongolia in the critical areas of this study. Of the 415 participants in this study all had previous pregnancies, 6.5% (n=27) of which resulted in DS or another congenital defect. For those women who had experienced a live birth with a congenital abnormality, this event became the primary motivating factor in seeking professional obstetric care for their subsequent pregnancies. Prenatal care for these, and many of Mongolia's women, is little to none, often relying on family or friends for advice and guidance during this critical time. This study reinforces the value of professional prenatal care in Mongolia. Coupled with the Prenatal Biomarker Screening for Congenital Defects Care Program withinour Regional Diagnostic Centers and District Medical Health Centers, in Ulaanbaatar, Mongolia the study will serve to further expand support for pregnant women in Mongolia.

In this study, the sensitivity was calculated using a combination of maternal age, NTT, and precise double-test biomarkers, using a cut-off risk value of 1 in 300. For trisomy 21 the sensitivity was 66.6% and for all other types of aneuploidy was 80%, with a false positive rate of 2.2%. The results of our study are comparable with a detection rate of 93% and 95% for trisomy 21 and for all types of aneuploidy respectively. In one study population there was a false positive rate of 2.5 % [18]. Marius et al, observed a detection rate of 83.3% and 81.1% for trisomy 21 and for all types of aneuploidy respectively in a large study (n=2,339) on the population in the United States, with a false positive rate of 7.2% [19].

Our study result is also consistent with the findings of another large trial (n=3,900) on the sensitivity and false positive rates of this screening method [20]. There have been five prospective intervention studies that used both NTT and the first-trimester serum biomarkers PAPP-A and free β-hCG previously. Spencer et al, observed detection and FPRs of 86% (n=7) and 6.7% (3,762 pregnancies); Krantz et al, found rates of 91% and 7.9% (n=5,223); Bindra et al, reported rates of 92% and 6.8% (n=14,200); Schuchter et al, reported rates of 86% and 5.2% (n=4,939); and Crossley et al, reported rates of 80% and 5% (n=17, 229) [21-24]. Niemimaa et al, observed detection and false-positive rates of 80% (5cases) and 5.4% (n=1,602 pregnancies) using this method [25]. The false-positive rates in some of these studies are higher than ours, using the same biomarkers, and the detection rates are also somewhat higher. This is probably due in part to our choice of 1 in 250 at term as the cut-off, whereas they have generally used 1 in 270 or 300 during the first trimester. The relatively small number of affected pregnancies will also contribute to the between-study differences in detection rates. The landmark French National Screening Programme was implemented in January 1997 using second-trimester serum markers. A review of the 5,694 women screened in the first 2 years showed a study participation rate of 65%, with an observed DR of 73% and a 4.7 % FPR [26]. In the current non-intervention study, the combined use of NTT and serum biomarkers in the first trimester achieved a much lower false-positive rate 3.0% (n=19,614) and DR 89% [27]. Although the observed DR was not increased, at 67% the total number of DS cases was small and the model derivation rate was much higher at 91% [28].

Our work was directed at finding a more accurate and minimally invasive method for early diagnosis of trisomy 21 (DS), a genetic condition of concern in Mongolia. At present, the most common methods for detecting fetal genetic aneuploidies are to use ultrasound for fetal NTT, measurement of fetal crown-rump-length (CRL), and nasal bone characteristics during the first trimester. Our study shows that the inclusion of maternal age and the following maternal serum biomarkers greatly increases the confidence and accuracy of an earlier diagnosis: PAPP-A, free βhCG, and ultrasound-determined NTT. In trisomy 21, during the first trimester of pregnancy, the maternal serum concentration of PAPP-A is decreased while free βhCG is increased. Within our study, a detection rate approaching 91.7%, and a false positive

rate of just 4.5%, were obtained, thus greatly enhancing the certainty of an early diagnosis of DS. All of these tests are conducted in the first trimester, thereby allowing for early detection of fetal aneuploidies and the opportunity for more appropriate and beneficial genetic counseling for the parents.

**Limitations of this study and the validity of the measured parameters**

Limitations of our study include a modest population size of 415 participants. While it is reasonable to draw the conclusions that we did from this population, obviously a larger sample size would add further weight to the results we obtained. The fact that other, larger studies have been conducted in other countries and that our results are very similar to theirs gives us confidence in our results despite the sample size.

Application of our detection methods would certainly be appropriate for obstetric care throughout Mongolia. Coupled with other programs designed to improve the quality and outcomes of prenatal care within Mongolia, it would be reasonable to expect a notable improvement in the quality of care as well as access to care for pregnant Mongolian women.

**Conclusion and recommendations**

Our study results confirm the importance of prenatal screening and the use of cytogenetic studies in the identification of chromosomal abnormalities. These screening tests allow us to avoid potential harmful procedures for the mother and unaffected fetus. Prenatal cytogenetic findings are critical for proper genetic counseling and subsequent decision making. A maternal age of 35 years or older at the time of delivery should be used to identify women at high risk for having a child with trisomy 21 and/or other congenital birth defects. These women should be offered genetic counseling, prenatal screening, and diagnostic testing during their obstetric care. This study should also be referenced as a source of empirical scientific data and used to further the Prenatal Biomarker Screening for Congenital Defects Program withinour Two and Three Step Obstetric Clinical Care Program, Regional Diagnostic Centers and District Medical Health Centers, in Ulaanbaatar, Mongolia.

## Conflict of Interests

The authors have no conflicts of interest.

## Acknowledgements

This work was supported by the School of Medicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia.

The authors thank the National Center for Maternal and Child Health Laboratory and Genetic Counseling Cabinet in Ulaanbaatar, Mongolia for their assistance.

We also thank Mike Endres for his invaluable assistance with English grammar and language and statistical analysis translations.

## References

1. Benn PA, Fang M, Egan JF, Horne D, Collins R. Incorporation of inhibin-A in second-trimester screening for Down syndrome. Obstet Gynecol 2003; 101: 451-4.
2. Wapner R, Thom E, Simpson JL, Pergament E, Silver R, Filkins K, et al. First trimester screening for trisomies 21 and 18. N Engl J Med 2003; 349: 1405-13.
3. Brizot ML, Snijders RJ, Butler J. Maternal serum hCG and fetal nuchaltranslucency thickness for the prediction of fetal trisomies in the firsttrimester of pregnancy. Br J Obstet Gynaecol 1995; 102: 1227-32.
4. Spencer K, Ong C, Skentou H. Screening for trisomy 13 by fetalnuchal translucency and maternalserum free beta hCG and PAPP-A at 10–14 weeks of gestation. Prenat Diagn 2000; 20: 411-6.
5. Palomaki GE, Knight GJ, Donhowe JM. Maternal serum screening for Down Syndrome in the United States: a 1995 survey. Am J Obstet Gynecol 1997; 176: 1046-51.
6. Nicolaides KH, Azar G, Byrne D, Mansur C, Marks K. Fetal nuchal translucency ultrasound screening for chromosomal defects in first-trimester of pregnancy. BMJ 1992; 304: 867-9.
7. Nicolaides KH, Snijders RJ, Cuckle HS. Correct estimation of parameters for ultrasound nuchal translucency screening. Prenat Diag 1998; 18: 519-23.
8. Wald NJ, Hackshaw AK. Combining ultrasound and biochemistry in firsttrimester screening for Down's syndrome. Prenat Diagn 1997; 17: 821- 9.
9. Tabor A, Alfirevic Z. Update on procedure-related risks for prenatal diagnosis techniques. Fetal Diagn Ther 2010; 27: 1-7.

10. Wortelboer EJ, Koster MP, Stoutenbeek P, Elvers LH, Loeber JG. First-trimester Down syndrome screening performance in the Dutch population; how to achieve further improvement.Prenat Diagn 2009; 29: 588-92.
11. Cho CK, Shan SJ, Winsor EJ, Diamandis EP. Proteomics analysis of human amniotic fluid. Mol Cell Proteomics 2007; 6: 1406-15.
12. Pereira-Maxwell F. In: A–Z of Medical Statistics:A Companion for Critical Appraisal. London, UK: Arnold; 1998. P 75-77.
13. Lalkhen AG, McCluskey A. Clinical tests: sensitivity and specificity. Continuing Education in Anaesthesia. Critical care & Pain. Oxford, UK: Oxford University Press; 2008. p 221-223.
14. Brambati B, Simon G, Danesino C, Oldrini A. First trimester fetal diagnosis of genetic disorders: clinical evaluation of 250 cases. Am J Med Genet 1985; 22: 92-9.
15. Hook EB, Gross PK. Jackson L, Pergament E, Brambati B. Maternal age-specific rate of 47, 21 and other cytogenetic abnormalities diagnosed in the first trimester of pregnancy in Chorionic villus biopsy specimens: Comparison with rates expected from observations at amniocentesis. Am J Hum Genet 1988; 42: 797-807.
16. Pueschel SM. Clinical aspects of Down syndrome from infancy to Adulthood. Am J Med Genet 1990; 7: 52-6.
17. Anderson CL, Brown CL. Fetal chromosomal abnormalities: Antenatal Screening and Diagnosis. Am Fam Physician 2009; 79: 117-24.
18. Koster MP, Wortelboer IJ, Stoutenbeek P, Visser GH, Schielen PC. Modeling Down syndrome screening performance using first trimester serum markers. Ultrasound Obstet Gynecol 2010; 38: 134-9.
19. Chasen ST, Sharma G, Kalish RB, Chervenak FA. First-trimester screening for aneuploidy with fetal nuchal translucency in a United States population. Ultrasound Obstet Gynecol 2003; 22: 49-51.
20. Kublickas M, Grossly J, Aitken DA. Screening for Down's syndrome in the first trimester: Combined risk calculation, methodology, and validation of web-based system. AOGS 2009; 88: 16-20.
21. Krantz DA, Hallahan TW, Orlando F, Buchanan P, Larsen JW, Macri JN. First-trimester Down syndrome screening using dried blood biochemistry and nuchal translucency. Obstet Gynecol 2000; 96: 207-13.
22. Bindra R, Heath V, Liao A, Spencer K, Nicolaides KH. One stop clinic for assessment of risk for trisomy 21 at 11–14 weeks: a prospective study of 15,030 pregnancies. Ultrasound Obstet Gynecol 2002; 20: 219-25.
23. Schuchter K, Hafner E, Stangl G, Metzenbauer M, Hofinger D, Philipp K. The first trimester 'combined test' for the detection of Down syndrome pregnancies in 4939 unselected pregnancies. Prenat Diagn 2002; 22: 211-5.
24. Crossley JA, Aitken DA, Cameron AD, McBride E, Connor JM. Combined ultrasound and biochemical screening for Down's syndrome in the first trimester: a Scottish multicentre study. BJOG 2002; 109: 667-76.
25. Niemimaa M, Suonpaa M, Perheentupa A, Seppälä M, Heinonen S, Laitinen P, et al. Evaluation of first trimester maternal serum and ultrasound screening for Down's syndrome in Eastern and Northern Finland. Eur J Hum Genet 2001; 9: 404-8.
26. Muller F, Benattar C, Audibert F, Roussel N, Dreux S. Cuckle H. First-trimester screening for Down syndrome in France combining fetal nuchal translucency measurement and biochemical markers. Prenat Diagn 2003; 23: 833-6.
27. Kagan KO, Cicero S, Staboulidou I, Wright D, Nicolaides KH. Fetal nasal bone in screening for trisomies 21, 18 and 13 and Turner syndrome at 11-13 weeks of gestation. Ultrasound Obstet Gynecol 2009; 33: 259-64.
28. Sniefa S, Amargandhi M, Bhupendra J, Moulali S, Kristine T. First trimester maternal serum screening using Biochemical markers PAPP-A and free βhCG for Down syndrome, Patau syndrome and Edward syndrome. Ind J Clin Biochem 2013; 28: 3-12.