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THE IMPORTANCE OF PRENATAL SCREENING AND PRENATAL DIAGNOSIS IN THE IDENTIFICATION OF NUMERICAL CHROMOSOMAL ABNORMALITIES

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ABSTRACT

Objectives and aim: The obstetric care of a pregnancy, as it is practiced today, includes non-invasive screening approaches as well as invasive procedures for a definitive prenatal diagnosis of fetal disorders, correlations between indicators for prenatal cytogenetic diagnosis, and results of chromosomal analysis made upon fetal cells. The aim is to introduce the prenatal invasive and non-invasive diagnostic methods during the first and second trimesters of pregnancy, thus providing early detection and prevention of chromosomal abnormalities and congenital developmental disorders within pregnancy outcomes in Mongolia. **Results:** The indications to perform prenatal cytogenetic diagnosis for numerical chromosomal abnormalities were abnormal biomarkers of double and triple testing, advanced maternal age, fetal abnormality detected through ultrasound, and positive obstetric history for chromosomal aneuploidy. The study identified 3 cases with abnormal numeric chromosomes of the two Downs cases showed karyotypes 47, XX, 21+, cytogenetic types of Down Syndrome (DS) and one case was karyotypes 47, XY, 13+, cytogenetic types of Patau Syndrome. **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 an effective prenatal screening and cytogenetic diagnosis for fetal aneuploidycan be applied to pregnancy outcomes in Mongolia.

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INTRODUCTION

Abnormal congenital development isone of the leading causes of infant mortality in Mongolia (19.4%) as well as in the world(National Health Development Center in Mongolia of Statistical source 2010).Prenatal screening and diagnosis for chromosomal aneuploidies and congenital developmental disorders has become an integral part of prenatal care worldwide (Anderson *et al.,* 2009, Pennings *et al.,* 2009, Reyolds, 2010).

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Methods of prenatal diagnosis can be divided into invasive and non-invasive techniques. Non-invasive methods include ultrasound and biochemical screening using maternal blood samplesWald *et al.,* 2003, Evans *et al.,* 2007,).

Non-invasive techniques: Screening is an effective method 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 beta human chorionic gonadotropin (βhCG) have been demonstrated in several large studies to have comparable or greater accuracy

than other methods (Benn *et al.,* 2003, Wapner *et al.,* 2003). In trisomy 21, during the first trimester of pregnancy, the maternal serum concentration of PAPP-A is decreased while free βhCG is increased (Brizot *et al.,* 1995, Spencer *et al.,* 2000). In trisomy 13 and 18 maternal serum concentration of PAPP-A and free βhCG are both decreased (Palomaki *et al.,* 1997).Studies have shown that NTT measurements between 11 and 14 weeks' gestation, when combined with maternal age, yield a detection rate (DR) of 75%, with a false positive rate (FPR) of just 5% (Nicolaides *et al.,* 1992).When these two parameters are combined with PAPP-A and free βhCG, the DR of chromosomal abnormalities can increase up to 85-90% with a FPR of just 5% (Nicolaides *et al.,* 1998). Second-trimester screening using three maternal serum biomarkers; alpha fetoprotein (AFP), human chorionic gonadotropin (HCG) and unconjugated estriol (uE3) are measured and combined with maternal age to estimate risks at term (Palomaki *et al.,* 1987).Second trimester screening is mostly carried out between gestation weeks 14 and 20. Within that period, the median of AFP and uE3 rise, with lower values being observed in trisomy 21. Medians of HCG decrease between weeks 14 and 20 while higher values are observed in trisomy 21(Graves *et al.,* 2002).Of all congenital anomalies, Open Neural Tube Detections (ONTD) are the easiest to identify prenatally(Boyd *et al.,* 1988).In the 1980s, maternal serum programs became available to identify pregnancies at risk for 75-90% of ONTDs and ≥ 95 of anencephaly can be detected by elevated maternal serum AFP values with a screen positive rate (PR) of 5% or less(Wald *et al.,* 1977). Second trimester ultrasonography may identify fetal anatomic defects, such as congenital heart defect or biomarkers suggestive of fetal aneuploidy such as nuchal translucency thickness, absent nasal bone, renal prereleases, or echogenic bowel (Kagan *et al.,* 2009; Boyd *et al.,* 2007; Chasen *et al.,* 2003; Al-Kouatly *et al.,* 2001). Ultrasonography is also used for screening in the second trimester, either alone or as an adjunct to maternal serum testing. Using this screening process some 75% to 85% of fetuses with DS could be detected, with a FPR of only 5%(Benn 2003,Cuckle *et al.,* 2005, Wright *et al.,* 2007).Therefore, it is reasonable to conclude that using these non-invasive prenatal methods, more accurate tests that provide a high DR and a relatively low FPR are 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 so indicated (Cho *et al.,* 2007).

Invasive techniques: The use of invasive techniques for prenatal diagnosis of chromosomal abnormalities includes such procedures as amniocentesis and CVS(Ogilvie, 2003).CVS is performed in the first trimester between 10 and 13 weeks' gestation, while amniocentesis can be performed as early as 15 weeks' gestation (Alfirevic *et al.,* 2003, Tabor *et al.,* 2009). Fetal chromosome analysis has been traditionally performed using G banding of cultured cells in metaphase and has long been considered to be the gold standard detection method (Nicolini *et al.,* 2004, Wapner 2005, Zhang *et al.,* 2009).This technique is accurate and reliable, thus allowing the detection of a variety of numerical and structural aberrations. The diagnostic accuracy of karyotyping with amniocentesis is 99.4 to 99.8% (JAMA, 1976)and for CVS 97.5 to 99.6%, providing a significant level of clinical confidence for the provider(Hahnemann *et al.,* 1997).In those countries where chromosomal abnormalities and birth control systems are present and applied in the clinical setting, prenatal screening and invasive diagnostic methods have been utilized to prevent the birth of a fetus and infant mortality does not exceed 15.0 per 1,000 infant mortalities due to chromosomal aberrations (Wortelboer *et al.,* 2008). In Mongolia, the fifth most common cause of infant mortality are congenital defects. Infant mortality due to congenital abnormalities has been steadily increasing from 17.8 in 2007 to 19.4 in 2010 (per 1,000 live births).These is still no confirmed national data on the prevalence of major aneuploidies within Mongolia, specifically for DS. An annual prevalence of DS of 0.9 in 1,000 births has been reported between the years 2004-2009. (The State Research Center on Maternal and Child Health, Mongolia 2009).According to surveys carried out by medical scientists in Mongolia, approximately 10% of nearly 700 children involved in outpatient genetic consultations have been diagnosed for DS with the pregnant mothers being between 34-45 years of age. In 2010 the Ministry of Health of Mongolia put a special emphasis on reducing infant and pre-schoolmortalities, seeking to reach a goal of 15.0 per 1,000 infant mortalities by 2015 (National Health Development Center in Mongolia of Statistical source 2010). Currently, Mongolia has not had a formal antenatal screening program conducted by medical officials, creating a void for any comprehensive prenatal screening for chromosomal abnormalities or congenital defects. The purpose of this study was to introduce prenatal diagnostic technology in obstetric practice in Mongolia, thus raising the standard of care for all pregnant women.

MATERIALS AND METHODS

The obstetric history was recorded and serum biomarkers were measured in 1,096pregnant women receiving obstetric care at the National Center for Maternal and Child Health Laboratory and Genetic Counseling Cabinet in Ulaanbaatar, Mongolia. The research was directed at establishing a single laboratory data base of women with an obstetric history of pregnancies between November 2010and January 2014. Maternal blood serum biomarkers were measured in 1,096 women with pregnancies in the first and second trimesters. The pregnancies were separated into five age groups: than less 24 years, 25-29, 30-34, 35-39 years, and more 40.The project study was conducted using three categories: first trimester screening (FTS), second trimester screening (STS), and combined screening groups with an emphasis on DS, along with other congenital birth defects. In those cases, that were shown to be positive and high risk, CVS and AFC as well as further cytogenetic analysis were carried out.

1st step: In the first trimester, for all 415 pregnancies with gestational ages between 11 to 13-weeks, maternal serum biomarkers were double tested for PAPP-A and β-hCG and measured for fetal NTT. In the second trimester, 681 pregnancies with gestational ages between 16 to 21weeks, maternal serum biomarkers were triple tested for AFP, HCG, and uE3 biomarker mean and median values. The intent was to identify those baseline characteristics associated with all pregnant Mongolian women during this period.

2nd step: Noninvasive screening, using the multiple serum biomarkers, then revealed those women at increased risk of carrying a fetus with risk trisomy 21 and other fetal aneuploidy and congenital defects at birth. The results of fetal NTT in the first and second trimester using maternal serum biochemical markers along with maternal age were then entered into Fetal

Medicine Foundation (FMF) software and a positive risk assessment was then calculated.

3rd step: Women who were found to be at increased risk of carrying a fetus with Trisomy 21 or other congenital defects to full term were offered counseling and the option to do further, invasive testing. A total of30 were further identified as being at high-risk for congenital anomalies using G-banding of 28 samples of AFC and CVS were used for further genetic diagnosis.

Laboratory MeasurementsSpecimen collection and preparation: or 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 were subsequently kept frozen at -70^0 C. Repeated freeze-thaw cycles of the 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, βhCG, AFP, HCG and uE3 analyzed with enzyme-linked immunosorbent assay (ELISA) using kits from Dynex Technologies 2®. DRG International, Inc. The micro-titer wells (kits) are coated with a polyclonal anti PAPP, βhCG, AFP, HCG and uE3 antibody. An aliquot of the patient sample containing PAPP, βhCG, AFP, HCG and uE3 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 FMFtrained ultra-sonographer adhering to standardized protocols.

Laboratory Cytogenetics method: There are a number of options for diagnostic tests on cells obtained from CVS or amniocentesis including: Investigation for chromosomal anomalies was performed using routine cytogenetics analysis G-banding. Cytogenetic diagnosis has been done in chromosome preparations of leukocytes cultured from peripheral chorionic villi sampling and amniotic cells according to a modification of the technique used by Hungerford(Moorhead 1960, Hungerford 1965).The traditional standard of diagnosing prenatal chromosomal abnormalities using metaphase analysis via the G-banding by Seabright was used (Seabright 1971). However, the Ikaros 5.5 Demo Tutororial meta-system software has proven to be a more accurate and reliable method for analyzing chromosomal abnormalities.

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) (Pereira-Maxwell 1998, Lalkhen *et al.,* 2008).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 isthe false positive rate (Pereira-Maxwell 1998, Lalkhen *et al.,* 2008).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 substance from the standard curve is the LLD. This value represents the smallest amount of a substance can be distinguished from the abscense 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 regressed medians were calculated using a log-linear relationship of the first degree. The biomarkers were transformed to $\log_{10}$ data and the weeks were on an arithmetic scale. 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 and AFC. The sensitivity & specificity analysis was done using Clinical Decision Making Program software from the Department of Family Medicine, University of Oklahoma Health Sciences Center and Microsoft Excel, SPSS 20, and STATA 14.2 software.

Ethics: 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 1,096 participants within the study group,15 were diagnosed with twins. The mean maternal age was 32.1±6.50 (range of 18-49years), while 454 participants were over 35 years of age (41.4%). The mean maternal body weight, in kilograms, was 67.2±10.1 (range of 44-125 kg). All study participants had at least one previous pregnancy. Of these, 313 pregnancies resulted in miscarriages (28.5%), 117 women experienced in utero fetal demise (10.6%),there were 99 premature births (9.03%), and 70 stillbirths (6.38%).These events were the motivating consideration for 632 (57.6%)women to seek professional obstetric care during their subsequent pregnancy. One hundred forty women (12.7%) smoked during the pregnancy, twenty-five (2.28 %) were occasional smokers, and nine hundred twenty-one(84.9%)were non-smokers. Five hundred thirty-five (48.8%) were exposed to second-hand smoke.

Elevated maternal serum biomarkers values in pregnancies and fetal NTT: The comparison revealed that there is significant ($p<0.05$) difference in medians of each gestation

compared to the overall median of PAPP-A, βhCG, AFP, HCG, uE3 and NTT. The median equations were established by least squares regression of logarithmic-transformed biomarker values gestational age. PAPP-A and uE3 results were expressed in nmol/l; and βhCG, HCG in mIU/ml; AFP in IU/ml and NTT in mm. In first trimester of the 415 pregnancies studied, 403 (97.1%) screened negative while 12 (2.9%) screened positive. Of the 681 pregnancies studied, 606 (89.0%) screened negative while 75 (11.0%) screened positive in second trimester. During the first and second trimester for all pregnancies, the mean serum biomarkers of the screen-negative group was PAPP-A 11.1±5.2ng/ml ($p<0.001$), βhCG 36.8±21.6mIU/ml, HCG 34.8±20.2 mIU/ml ($p<0.05$), AFP 47.4±25.4 ($p<0.001$), uE3 6.9±5.6 ($p=0.374$)and fetal NTT 0.19 ±0.07 ($p<0.001$). The median values concentration of screen-negative women was calculated 10.0 ng/ml PAPP-A, 32.2 mIU/ml βhCG, 43.7 IU/ml AFP, 19.3 mIU/mlHCG and 4.5nmol/l uE3 and 1.8 mm fetal NTT. It can be seen that AFP and uE3 biomarkers both increasing and HCG decreases with gestational age throughout the period under study. The screen-negative group data were expressed as the median values concentration (P2.5-P97.5) and had significant (95%) confidence intervals (CI) of 10.6 to 11.6 PAPP-A, CI 34.6 to 38.9 βhCG, and CI 1.8 to 1.9 fetal NTT in the first trimester and CIof 45.4 to 49.5 AFP, CI 31.4 to 38.1 HCG, CI 6.2 to 7.5 uE3 in the second trimester. The number of screened positive for maternal age with abnormal fetus that were less than 35 years of age was sixty-one women (9.50%) while over 35 years of age, there were twenty-six women (5.72%).The first and second trimester of 87 cases that screened positive for aneuploidies and congenital defects using PAPP-A, βhCG, AFP, HCG and uE3 had below median values.

The mean serum biomarkers of the group that screened positivein the first and second trimester were 6.6±3.3 ng/ml PAPP-A ($p<0.002$), 28.9±18.1 mIU/ml βhCG ($p<0.05$), 65.3±49.4 IU/ml AFP($p<0.001$), 34.8 ± 29.7mIU/ml HCG ($p<0.05$), 6.9±5.2 nmol/l uE3 and fetal 2.6±1.2 mm NTT($p<0.002$). The median values concentration of screen-positive women was calculated at 7.0 ng/ml PAPP-A, 26.9 mIU/ml βhCG, 54.9 IU/ml AFP, 25.5 mIU/ml HCG, 5.6 ng/ml uE3 and 2.4 mm NTT.The combined biomarkers from the group that screened positive were expressed as the median with (95%) confidence intervals (CI) of 4.5 to 8.7 PAPP-A, CI 17.1 to 40.4 βhCG, CI 54.0 to 76.7 AFP, CI 26.4 to 50.4 HCG, CI 4.8 to 7.2 uE3 and CI 1.9 to 3.4 fetal NTT in the first-second trimester and are shown in Table 1. 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% CI 21.1 to 78.9 and FPR 6.7% CI 4.5 to 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 all the women in three of these age groups were at increased risk for an abnormal fetus due to above normal NTT measurements (greater than1.6 mm to 1.9 mm).

Calculation of the sensitivity and specificity of the combined screening test for fetal aneuploidies: The method for calculating first trimester PAPP-A, ß hCG with NTT and second trimester total HCG, uE3, AFP, and maternal age

Table 1. In the first and second trimester biomarkers median values for each gestation weeks determined of SCREEN-POSITIVE GROUP

Median value and 95% confidence intervals (CI) in the first trimester							
Gestational week	**N samples**	**PAPP-A (ng/ml)**	**95% CI**	**BhCG (mIU/ml)**	**95% CI**	**NTT (mm)**	**95% CI**
11	2	3.3	3.2 - 3.2	41.2	34.4 - 48.0	1.6	-0.9 - 4.1
12	6	7.0	3.3 - 10.1	25.4	14.2 - 31.2	2.4	1.3 - 3.8
13	4	8.5	2.3 - 13.8	21.0	-16.0 - 79.8	3.8	1.5 - 5.0
Total	12	7.0	4.5 - 8.7	26.9	17.4 - 40.4	2.4	1.9 - 3.4
Median value and 95% confidence intervals (CI) in the second trimester							
Gestational week	**N samples**	**AFP (IU/ml)**	**95% CI**	**HCG (mIU/ml)**	**95% CI**	**uE3 (nmol/l)**	**95% CI**
16	7	28.1	14.1 – 74.7	25.1	0.6 – 36.9	2.0	1.0 - 4.9
17	4	41.1	-32 -164.1	20.9	-7.0- 61.5	4.4	0.9 - 11.2
18	8	46.7	35.1 - 44.8	21.3	1.1 -113.5	3.2	1.6 - 6.5
19	12	48.5	36.7 - 79.5	19.1	4.4 -124.1	3.6	2.6 - 6.2
20	22	58.4	52.1 - 85.2	27.5	20.4 - 43.5	4.7	4.0 - 9.2
21	22	58.9	50.0 - 111.8	17.3	10.8 - 49.8	7.1	5.4 -11.0
Total	75	54.9	54.0 - 76.7	25.5	26.4 - 50.4	5.6	4.8 - 7.2

The positive group data expressed as a medians obtained with a simple log-liner relationship and (P2.5-P97.5) with were significant and 95% confidence intervals.AFP=alpha fetoprotein, hCG=human chorionic gonadotropin, uE3=unconjugated estriol

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

Table 2. Correlations between the screening test results and prenatal diagnosis results of fetal aneuploidies

	Screening test result multiple of median (MoM) and mean									Cytogenetic analysis result	
	N	**MA**	**PAPP-A**	**βhCG**	**NTT**	**MA**	**AFP**	**HCC**	**uE3**		**Karyotype**
T21	2	35	0.24	1.27	2.5	43	0.19	1.34	0.32	CVS/AFC	47, XX, 21+
			3.20	46.3	3.9		6.61	52.4	1.9		47, XX, 21+
T13	1	30	0.92	1.29	1.0		-	-	-	CVS	47, XY, 13+
			6.21	29.5	1.8						

trisomy 21 (T21), trisomy 13 (T13), maternal age (MA), chorionic villus sampling (CVS) and amniotic fluid cells (AFC). The measurement unit's ng/ml PAPP-A, nmol/l uE3, IU/ml AFP, mIU/ml βhCG and HCG and mm –NTT

The estimated high risk was calculated by the FMF software for trisomy 21 (The background risk 1:58 for aneuploidies is based on the maternal ages 35 and 43 years. 1:288 is adjusted risk in first trimester risk of biomarkers values calculated on the basis of background risk and ultrasound factors).At each weeks 12; 13 and 16 weeks in cytogenetic diagnosis.

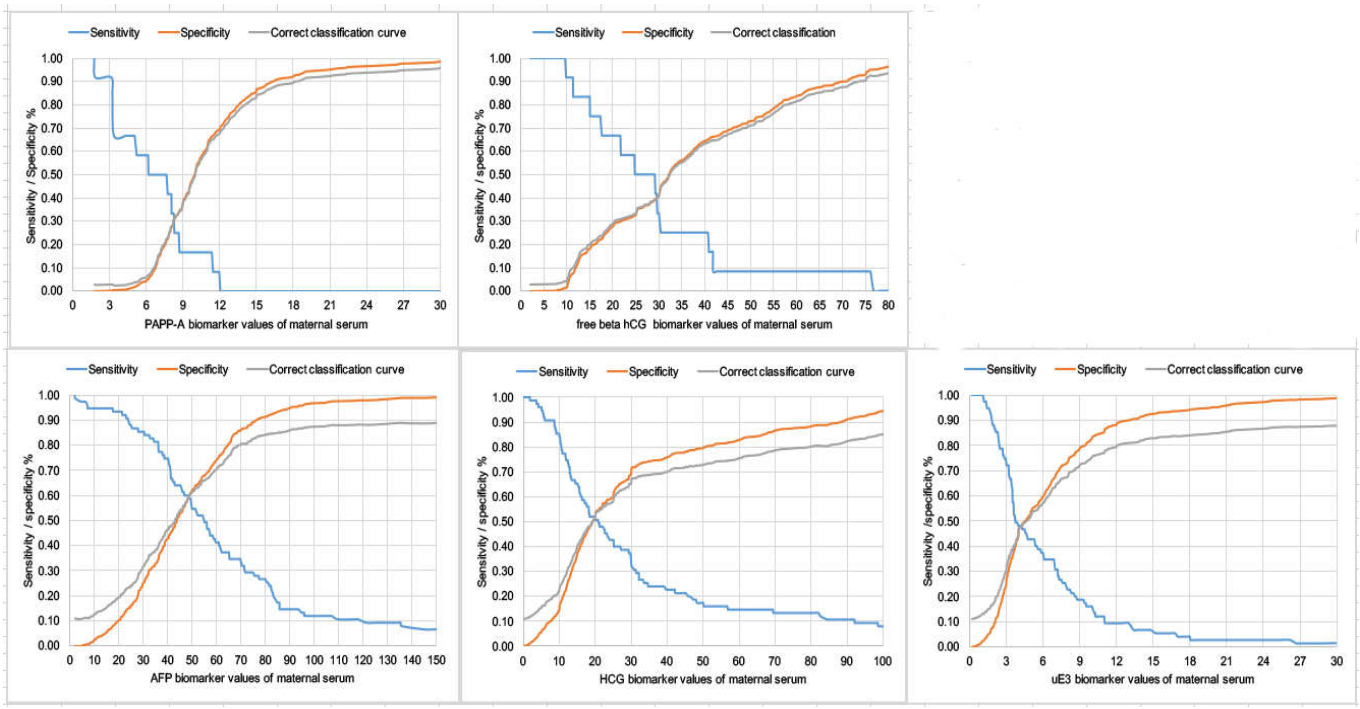

Figure 1. Calculations of sensitivity, specificity of screening of double and triple test biomarkers for all pregnancies and the correct classification curve for fetal abnormalities. Sensitivity and specificity of PAPP-A, free beta hCG, AFP, total HCG, uE3

evaluated biomarkers sensitivity of the screening test for fetal trisomy 21 was 75.0 % and for all fetal aneuploidy was 83.3% (CI 51.6 to 97.9). Specificity of the screening test for fetal chromosomal and congenital defects were 96.5% and the false positive rate was 3.9% (CI) 1.2 to 4.5 of fetal NTT screened.

Cytogenetic diagnostic results: Of the 1,096 pregnanies screened by biomarker first and second tests, 1,009(92.07%) were screen negative and 87(7.93%) pregnancies were screen positive. A total of 30 cases of high-risk for fetal abnormalities underwent further invasive prenatal testing for karyotyping for confirmation. These high-risk cases were additionally investigated using G-banding for the 28 cases at 16 to 21 weeks' gestation for AFC was one casetrisomy 21 and at 12 to 13 weeks' gestation. Two cases of CVS for genetic diagnosis trisomy 21 and 13 were found. 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 the women's first trimester. Table 2 shows the observed median levels in the Down syndrome pregnancies for maternal serum 0.24 MoM PAPP-A, 1.27 MoM β-hCG,0.19 AFP and2.5 NTT MoM. Regarding the gestational age, the amniocentesis was performed between 16 and 21 weeks of gestation. The most frequent indications of amniocentesis were abnormal maternal screening, advanced maternal age, abnormal ultra-sonographic finding and a family history of abnormalities. This high risk group analyzed both the maternal screening and the abnormalities in karyotype of the pregnancies with trisomy 21 was higher for the pregnant mothers that were in the 35-39 and 40-45 year-old groups. The cytogenetic analysis result of the two cases with DS were characterized by an extra chromosome 21. The fetal karyotype of each was 47, XX 21+. In the case of other chromosomal abnormalities, trisomy 13 was found in the pregnant mothers who were 26-30 years old. That one case was Patau syndrome, characterized by an extra chromosome 13 and karyotype was 47, XX 13+. The other 27 cases with other congenital malformation defects birth of a normal karyotype were found by AF sampling in Table 2. For routine cytogenetic analysis however the G-banding technique using trypsin and chromosomes with Giemsa solution became the most frequently used methods for karyotyping result of gender was 18(60%) female and 12(40%) males.

DISCUSSION

Our study, providing a comprehensive set of diagnostic and diagnostic procedures for the first and second three months of prenatal care for chromosomal abnormalities, early detection and diagnosis of congenital malformations, diagnosis, prevention and genetic counseling in Mongolia, is the first such study within the practice of maternal and fetal medicine. DS results in the most common chromosomal disorder in humans and is present in approximately one out of 500-800 live born children (Egan *et al.,* 2004). Prenatal screening for DS usually consists of risk calculation based on biochemical and biometric parameters, as well as maternal age, after which women with a high predictive risk may opt for invasive testing, such as amniocentesis or chorion villus sampling (Cuckle *et al.,* 1987, Wald *et al.,* 1988).

First and second trimester screening of non-invasive techniques: Our intent was to determine biomarkers median values and the sensitivity and specificity of a double and triple test method using the combination of maternal age and NTT for all fetal aneuploidy including DS and other detection congenital. While there are other, similar, studies in the literature, none address the distinctive population Mongolia in the critical areas of this study. Of the 1,096 participants in this study all had previous pregnancies, 57.6% (n=632) 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. Multiple with the Prenatal Biomarker Screening for Congenital Defects Care Program within our Regional Diagnostic Centers and District Medical Health Centers, in Ulaanbaatar, Mongolia the study willserve 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 biomarkers of double and triple-test, using a cut-off risk value of 1 in 300. Our study results first trimester PAPP-A, ßhCG with NTT and second trimester total HCG, uE3, AFP, and maternal age evaluated biomarkers sensitivity of the screening test for fetal trisomy 21 was 72% 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 96.5% and the false positive rate was 3.9% (CI 1.2 to 4.5) of Fetal NTT screened. Our study result is also consistent with the findings of another large trial studies trial first trimester PAPP-A and second trimester total hCG, uE3, AFP and PAPP-A, and maternal age evaluated estimated a sensitivity of 78% (CI) 66 to 86 and specificity of 98% (CI) 96 to 99 at a cut-point of 1:200 risk(Wright *et al.,* 2010). First trimester PAPP-A and second trimester total hCG, uE3, AFP and inhibin A, and maternal age evaluated in three studies estimated a sensitivity of 87% (CI) 81 to 91 at a cut-point of 5% FPR(Malone *et al.,* 2005,Palomaki *et al.,* 2006).First trimester NT and second trimester free ßhCG and AFP, and maternal age evaluated in two studies estimated a sensitivity of 83% (CI) 70 to 91 at a cut-point of 5% FPR (Rozenberg *et al.,* 2002, Wald *et al.,* 2003) reported detection rates of 71% and FPR 7.2 (Summers *et al.,* 2003). As part of the first and second-trimester Prenatal Screening Program, the pregnancy outcome is sought for all women in order to predict fetal chromosomal abnormalities. Using data collected from the prenatal screening and diagnostic program, eighty-seven (7.93%) positive cases with two (2.29%) cases of Down syndrome, one (1.14%)case of Patau syndrome, and eighty-four (5.64%) cases of congenital defect were identified in the study participants. From the total number of positive pregnancies (n=87), 3.44% women were pregnant with fetuses that had numerical chromosomal abnormalities. Some previous studies showed a higher incidence of numerical abnormalities 4.61% to 4.85% (Lim *et al.,* 2002; Pergament *et al.,* 2002) while others had results similar to ours (2.01%) (Benn, 2003).

Analyzing both the maternal age and the abnormalities in karyotype, we concluded that the incidence of trisomy 21 was higher for pregnant mothers that were in the 35-39 and 40-44 years' groups. Similar results were reported by(Sung-Hee Han *et al.,* 2008)2.17% for pregnant mothers that were 41-45 years old. This observation indicates that there the risk of trisomy 21 increases with the age of the mothers. 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, AFP, HCG, uE3 and without ultrasound marker determined NTT. In trisomy 21, during the first and second trimester of pregnancy, the maternal serum concentration of PAPP-A and AFP is decreased while hCG is increased. Within our study, a detection rate approaching 96.5%, and a false positive rate of just 3.9%, were obtained, thus greatly enhancing the certainty of an early diagnosis of DS. However, women who have a high risk screening test result, and given amniocentesis or CVS have a risk of miscarrying a baby unaffected by Down's. All of these technologies are conducted in the first and second trimester, thereby allowing for early detection of fetal aneuploidies and the opportunity for more appropriate and beneficial genetic counseling and diagnostic testing during their obstetric care for the parents.

Limitations of this Study & the Validity of the Measured Parameters: Limitations of our study include a modest population size of 1096 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. Prenatal diagnosis 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 within our 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.

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