Original Article

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Integrating multiple of the median values of serological markers with the risk cut-off value in Down syndrome screening

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Summary **To assess the predictive value of integrating multiple of the median (MOM) with the risk cut-off value for serological screening of Down syndrome. In this retrospective study, women with singleton pregnancies who underwent triple serological screening for Down syndrome were followed, and their screening results and pregnancy outcomes were recorded. The range of MoM value of each indicator was calculated, different protocols integrating various MoM values with the risk cut-off value were compared. A total of 120,269 women with singleton pregnancy were screened and included in the analysis, of those 52 fetuses were confirmed as trisomy-21 by amniocentesis chromosomal karyotyping. Using a risk cut-off value of 1:380, 8,809 samples tested positive and the screen positive rate was 7.32% (8,809/120,269). The normal reference ranges (5-95%) of the MoM value of AFP, β-hCG, and uE3 were 0.60-1.72, 0.43-2.21 and 0.60-1.58, respectively. The detection rate of each screening protocol integrating different MoM percentile values was between 75% and 79%, the positive rate was between 7% and 18%, and the false positive rate was between 7% and 18%. Protocol-6 which combined the screening risk cut-off value and β-hCG MoM ≥ 97.5% percentile is an optimal protocol with a relatively high detection rate (78.8%) and low false positive rate (8.2%). Integrating MoM values of serological indicators can appropriately increase detection rate when interpreting the results of Down syndrome screening.**

Keywords: Down syndrome, serological screening, multiple of the median, prediction

1. Introduction

Down syndrome, also known as trisomy-21 syndrome, is the most common aneuploid type with a reported incidence between 1/1000 and 1/650 (*1,2*). Serological screening is widely used in prenatal screening for Down syndrome for fetal risk assessment (*3*). For cases with high-risk serological screening results, further prenatal diagnosis is recommended for confirmation. The serological screening mainly includes quadruple test [AFP (alpha fetoprotein), β-hCG (human chorionic gonadotropin), uE3 and Inh-A (Inhibin-A)] and triple test (AFP, β-hCG and uE3) in the second trimester, and the latter is widely used in China (*4*).

Multiple of the median (MoM) value is referred to as the ratio of the actual measured value of the three markers over the normal median value of the corresponding gestational weeks (days). When interpreting the results of Down syndrome screening, there are different notions on whether or not to include MoM values of each serological indicator and no consensus has been reached yet. Some researchers suggest that AFP MoM ≤ 0.5 and β-hCG MoM≥2.5 can be used as suitable cut-off values for screening Down syndrome pregnancy (*5*). And others only use median of MoM (mMoM) as a quality control measure but do not include in the screening criteria (*6-8*). From our clinical experiences, we have found that MoM value has certain clinical significance. In this retrospective

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study, we mainly aimed to evaluate the clinical value of integrating different MoM percentile values with the risk cut-off value for serological screening of Down syndrome.

2. Materials and Methods

2.1. *Study population*

This retrospective study was conducted at the Obstetrics and Gynecology Hospital of Fudan University. A total of 122,671 pregnant women who underwent second trimester (14 weeks^{+0} to 21 weeks^{+6}) triple serological test (AFP, β-hCG, and uE3) for Down syndrome screening during the period of June 2007 to July 2016 were included. Of those women, 120,269 women were with singleton pregnancy. Gestational week was confirmed with the last menstruation period and fetal crown-rump length (CRL). The pregnancy outcome of all those women was followed up.

The study protocol conformed to the ethical guidelines of the 2000 Declaration of Helsinki and was approved by the institutional review board at Obstetrics and Gynecology Hospital of Fudan University. Oral informed consent was obtained from all participants.

2.2. *Serological screening*

For each pregnant woman, 2 ml of maternal peripheral blood was extracted, and conserved at 4℃ after separation of serum. Serological markers (AFP, β-hCG and uE3) were detected within 3 days using chemiluminescent immunoassay (*9*). Instruments and reagents were provided by BECKMAN COULTER (USA). Chemiluminescence detection was performed using Beckman Coulter Access2 Automatic Immunoassay System (BECKMAN COULTER, USA) according to the manufacturer's instructions.

After chemiluminescent immunoassay, TCsoft prenatal screening software was used to calculate the screening risk. Values included in the software were serological markers (AFP, β-hCG and uE3), birthday of the mother, gestational weeks, height, weight, history of pregnancy, and fetal numbers (*10*). It automatically calculated the risk of Down syndrome, Open spina bifida (OSB) and Trisomy 18 syndrome (Final risk = age related risk* AFP likelihood ratio* β-hCG likelihood ratio* uE3 likelihood ratio). Using the software, the cut-off value for Down syndrome risk was set at 1:380 (*11*).

2.3. *MoM calculation and correction for each serological marker*

MoM value is the ratio of the actual measured value of the indicator to the normal median of the gestational weeks (days). In order to eliminate the influence of gestational weeks on markers when calculating the screening risk of Down syndrome, the MoM values of various serological indicators were calculated (*12*). In addition, a quality control system based on the mMoM was utilized to control the quality of the prenatal screening for Down syndrome. The ideal mMoM value should be within the range of 1.0 ± 0.05 (*7,8*). The mMoM values of the three markers (AFP, β-hCG, uE3) in our hospital in the last decade were 0.83, 1.00, and 1.00 respectively. The mMoM value of the AFP marker was significantly off 1.00 (median = 0.83, range: 0.73-0.94). In order to control for systematic error, all MoM AFP values were divided by 0.83 to correct for this deviation (*6,13*).

2.4. *Statistical analyses*

Mean and standard deviation (SD) were calculated for continuous variables, and were compared using Student's *t* test. Number and frequency were calculated for categorical variables, and were compared using Chi-square tests. Wilcoxon rank sum test was used to compare MoM values of each serological marker. Positive rate (screening positive cases/total cases), detection rate (true positive cases detected/true positive cases total), false positive rate (false positive cases/true negative cases total), positive predictive value (true positive cases detected/total positive cases detected) were calculated for each screening protocol.

All above analyses were two sided and were performed by SPSS 16.0 for Windows (SPSS Inc., Chicago, IL, USA). A P value of < 0.05 was considered statistically significant.

3. Results

3.1. *Population characteristics*

A total of 120,269 women with singleton pregnancies were included in the final analysis. Of those screened, 52 fetuses were confirmed as trisomy-21 by chromosomal karyotyping during amniocentesis or blood test after birth. The prevalence rate of Down syndrome in our population was 0.04% (52/120,269). Table 1 shows the population characteristics of the two groups. Compared to women without Down syndrome pregnancies, the average age of pregnant women in the Down syndrome pregnancy group was significantly older (31.15 ± 4.17 *vs*. 29.23 ± 3.44, $P < 0.05$). There was no statistically significant difference in weight, gestational age, smoking and gestational diabetes status between the two groups ($P > 0.05$ for all).

3.2. *Results of screening and follow-up outcome*

As shown in Table 2, using a risk cut-off value of greater than 1:380 (*11,13*), 8,809 samples tested

Table 1. Characters of the Down syndrome pregnancy group and the group without Down syndrome pregnancy

Items	Down syndrome pregnancy group (N = 52)	Control group (N = 120,217)	P
Age (years), Mean ± SD	31.15 ± 4.21	29.23 ± 3.44	< 0.001
AMA			
Yes	8	6,324	0.001
No	44	113,875	
Weight (kg)	57.32 ± 7.77	57.91 ± 8.88	
Gestational age (weeks)	16.65 ± 0.99	16.89 ± 1.27	0.63
Smoking			0.18
Yes	1	1,692	0.75
No	51	118,525	
GDM			
Yes	0	533	0.63
No	52	119,684	

AMA, advanced maternal age, defined as ≥ 35 years of age; GDM, gestational diabetes mellitus; SD, standard deviation.

Table 2. Second trimester serological screening results of Down syndrome

Screening	Down syndrome pregnancy group	Control group	Total
Positive (≥ 1:380)	39	8,770	8,809
Negative (< 1:380)	13	111,447	111,460
Total	52	120,217	120,269

Table 3. Percentile distribution of the MoM values of each indicator in 120,269 samples

Percentile (%)	AFP MoM	β-hCG MoM	uE3 MoM
2.5	0.550	0.357	0.540
5	0.604	0.426	0.603
50	0.995	0.994	0.999
95	1.719	2.213	1.576
97.5	1.930	2.603	1.718

AFP, alpha fetoprotein; β-hCG, human chorionic gonadotropin; MoM, multiple of median.

positive among 120,269 samples and the screen positive rate was 7.32% (8,809/120,269). Of the 8,809 samples, 39 (39/8,809, 0.4%) cases of Down syndrome were confirmed. Among the screening negative women, 13 were false negative cases classified using serological screening risk cut-off value of Down syndrome.

3.3. *Distribution of MoM values for each indicator*

Table 3 lists the different percentile distributions of the MoM values for each indicator. Compared to the control group, the Down syndrome pregnancy group had a higher β-hCG value, but lower AFP and uE3 values ($P < 0.05$ for all).

3.4. *Down syndrome cases*

Among the 52 women with Down syndrome pregnancies, one was a smoker, while none of them had gestational diabetes. Ten cases (10/52, 19.23%) were delivered, while 42 cases (42/52, 80.77%) were confirmed by amniocentesis chromosomal karyotyping and underwent induced labor. Eight of the 10 (8/10, 80%) live birth Down syndrome cases were screened as low risk using the risk cut-off value of less than 1:380, another 2 (2/10, 20%) cases were classified as high screening risk but refused amniocentesis. The detailed information of 10 cases of live birth Down syndrome cases is presented in Table 4.

Thirty-seven of the 42 (37/42, 88.10%) cases that underwent induced labor were screened as high risk (risk cut-off value of ≥ 1:380) and received amniocentesis chromosomal karyotyping; another 5 cases (5/42, 11.90%) were screened as low risk using the risk cut-off value of less than 1:380 but ultrasonographic screening indicated fetal abnormality, and were then confirmed by amniocentesis chromosomal karyotyping (Table 5). In total, twenty-six of the 52 (26/52, 50%) confirmed cases were detected with soft ultrasound markers for Down syndrome, including 6 cases of absent or hypoplastic nasal bone, 6 cases of nuchal fold thickness ≥ 6 mm, 5 cases of ventricular septal defect, 3 cases of ventriculomegaly, 2 cases of dilation of the renal pelvis, and 4 cases with multiple risk markers.

3.5. *False negative cases*

Among the confirmed 52 Down syndrome cases, 13 (13/52, 25%) were classified as false negative cases using serological screening of Down syndrome. The information of these 13 cases is shown in Table 6. The average age of the false negative cases was less than 35 (mean = 29.97, SD = 2.96). The risk of Down syndrome was between 1:1423 and 1:475. The MoM value of AFP was less than 0.7 for 4 cases, the MoM value of β-hCG was more than 2.0 for 2 cases, and the MoM value of uE3 was less than 0.7 for 2 cases. Five of the 13 (5/13, 38.46%) false negative cases underwent amniocentesis chromosomal karyotyping after ultrasound indication of fetal anomalies and then underwent induced labor, while the other 8 cases were delivered.

Table 4. Information of the 10 live birth Down syndrome cases

Case Number	AFP MoM	β-hCG MoM	uE3 MoM	Gestational age (week)	Weight (kg)	Down syndrome risk	Maternal age (year)	Clinical information						
								Ultrasound	Gestational age at delivery (week)	Delivery mode	Sex of newborn	Weight of newborn (g)	Chromosome examination	Outcome
1	0.62	1.1	0.93	15.4	66	345	35.55	Nasal bone hypoplasia	37.1	CS	Male	3065	Trisomy-21	Live
2	1.03	1.47	0.65	15.4	57	508	30.35	Ventricular septal defect	38.2	VD	Male	3450	Trisomy-21	Live
3	1.18	1.37	0.72	15.4	66	730	31.85	-	39.5	VD	Male	3660	Trisomy-21	Live
4	1.01	0.91	0.94	15.6	55	3601	26.58	Placental previa	36.3	CS	Male	2380	Trisomy-21	Dead
5	0.59	2.5	0.84	16.3	63	169	26.66	-	32.2	VD	Male	1795	Trisomy-21	Dead
6	0.63	1.69	0.83	16.1	54	435	23.89	-	37.4	VD	Female	2530	Trisomy-21	Live
7	0.75	1.05	0.99	16.1	49	754	34.49	-	38.3	CS	Female	3120	Trisomy-21	Live
8	0.87	1.26	0.79	17.6	50	391	34.48	Breech fetus	37.6	CS	Female	3010	Trisomy-21	Live
9	0.88	1.99	1.06	17.6	72	787	29.53	-	40.0	VD	Female	4050	Trisomy-21	Live
10	1.11	1.06	0.65	17.6	53	1380	29.23	Breech fetus	37.6	CS	Male	2830	Trisomy-21	Live

AFP, alpha fetoprotein; β-hCG, human chorionic gonadotropin; CS, cesarean section; MoM, multiple of median; VD, vaginal delivery.

Table 5. Down syndrome screening results and pregnancy outcomes

Items	Induced labor	Delivery	Total
High risk (< 1:380)	37	2	39
Low risk (≥ 1:380)	5	8	13
Total	42	10	52

3.6. *Comparison of different screening protocols*

We integrated the risk cut-off value with different combinations of the serological marker MoM percentile values to form different screening protocols, and summarized positive rate, detection rate, false positive rate, and positive predictive value of each protocol (Table 7). In general, integrating the risk cut-off value with different MoM percentile values of each indicator improved the detection rate of Down syndrome up to 79%.

Generally, the positive rate and false positive rate for most of the screening protocols were below 20%. It is observed that two protocols (3 and 6), which combined the screening risk and serological β-hCG MoM value, increased the detection rate to almost 80% while did not significantly increase the positive rate and false positive rate (both below 10%).

4. Discussion

The diagnosis of Down syndrome is mainly dependent on invasive prenatal examination and postpartum chromosome examination. The reported pregnancy loss rate for invasive prenatal diagnosis is between 0.3% and 1%, and varies depending on the skill and experience of the operators and specialist centers (*14*). Therefore, it is necessary to improve the detection rate of prenatal screening and reduce the false positive rate, thus reducing the invasive prenatal diagnostic procedure. Current prenatal screening is divided into early pregnancy screening and mid-pregnancy screening, based on the conditions and initial diagnostic time window of the medical institutions where the pregnant woman receives prenatal care. During the early pregnancy (11 weeks to 13^{+6} weeks), evaluation of Down syndrome risk is often based on the comprehensive analysis of maternal age, fetal neck translucency (NT) thickness, serum β-hCG and pregnancy-associated plasma protein A (PAPP-A). The detection rate of early pregnancy screening is between 80% and 82%, with a false positive rate of 3% (*14*). During the second trimester, serological triple (AFP, β-hCG, uE3) or quadruple (AFP, β-hCG, uE3 and Inh A) test integrating computation of maternal age risk is commonly used. Studies from other countries have reported that the quadruple test performed better than the triple test, with a detection rate close to 80%, and a false positive rate of 3% (*15,16*). However, in most parts

Table 6. Information of the 13 false negative cases

Case number	Gestational age (week)	Age (year)	Age risk	Risk of Down syndrome	AFP MoM	β-hCG MoM	uE3 MoM	Pregnancy outcome	Ultrasound
1	15.4	30.35	1:930	1:508	0.85	1.47	0.65	Delivered	Ventricular septal defect
2	15.4	31.85	1:760	1:730	0.98	1.37	0.72	Delivered	-
3	15.6	26.58	1:1,282	1:3,601	0.84	0.91	0.94	Delivered	-
4	16.1	23.89	1:1,423	1:435	0.52	1.69	0.83	Delivered	-
5	16.5	29.29	1:1,043	1:609	0.76	3.68	1.25	Induced labor	Nuchal fold thickness ≥ 6mm
6	16.1	34.49	1:477	1:754	0.63	1.05	0.99	Delivered	-
7	16.3	32.68	1:666	1:1,197	0.71	1.51	1.16	Induced labor	Nuchal fold thickness ≥ 6mm
8	16.5	29.00	1:1,071	1:3,980	0.93	1.29	1.19	Induced labor	Nuchal fold thickness ≥ 6mm
9	17.6	34.48	1:475	1:391	0.72	1.26	0.79	Delivered	Breech fetus
10	17.1	28.42	1:1,128	1:538	0.66	1.39	0.71	Induced labor	Nasal bone hypoplasia
11	17.6	29.53	1:1,014	1:787	0.73	1.99	1.06	Delivered	-
12	17.6	29.23	1:1,047	1:1,380	0.92	1.06	0.65	Delivered	Breech fetus
13	18.1	29.84	1:981	1:657	0.46	2.73	1.27	Induced labor	Head edema, tetralogy of fallot

AFP, alpha fetoprotein; β-hCG, human chorionic gonadotropin; MoM, multiple of median.

Table 7. Comparisons of different screening protocols

Number	Screening protocol	Positive rate (number of cases)	Detection rate	False positive rate	Positive predictive value
1	Risk cut-off value (1:380)	7.2% (8,614)	75.0% (39/52)	7.1%	1/221
2	Risk cut-off value + AFP MoM ≤ 5% (0.604)	11.3% (13,534)	76.9% (40/52)	11.2%	1/338
3	Risk cut-off value + β-hCG MoM ≥ 95% (2.213)	9.8% (11,733)	78.8% (41/52)	9.7%	1/286
4	Risk cut-off value + uE3 MoM ≤ 5% (0.603)	9.8% (11,733)	75.0% (39/52)	10.5%	1/324
5	Risk cut-off value + AFP MoM ≤ 2.5% (0.550)	9.2% (11,015)	75.0% (39/52)	9.1%	1/282
6	Risk cut-off value + β-hCG MoM ≥ 97.5% (2.603)	8.2% (9,910)	78.8% (41/52)	8.2%	1/242
7	Risk cut-off value + uE3 MoM ≤ 2.5% (0.540)	8.8% (10,536)	75.0% (39/52)	8.7%	1/270
8	Risk cut-off value + AFP MoM ≤ 0.7	17.8% (21,406)	78.8% (41/52)	17.8%	1/522
9	Risk cut-off value + β-hCG MoM ≥ 2.0	11.5% (13,807)	78.8% (41/52)	11.4%	1/337
10	Risk cut-off value + uE3 MoM ≤ 0.7	15.7% (18,860)	78.8% (41/52)	15.7%	1/460

AFP, alpha fetoprotein; β-hCG, human chorionic gonadotropin; MoM, multiple of median.

of China, early pregnancy screening rate is low because of missing time or technical limitations (*17*). The main method of screening fetal chromosomal abnormality in China is serological triple test, and further diagnostic procedures are recommended for high-risk pregnant women. Down syndrome screening in second trimester is a simple procedure with relatively low cost, which can be widely applied in China especially in less developed and resource limited regions.

It was reported that in the early time of second trimester, the AFP value of normal pregnant women had increased by 15-20% per week, serum uE3 value had increased by 20-25% per week, while serum β-hCG had decreased from the peak at 15 weeks and decreased slowly after 20 weeks (*18*). An early study reported that the AFP MoM of Down syndrome pregnancy was under 1.0 (*19*). A subsequent study found that the serum β-hCG of Down syndrome pregnancy was more than twice that of normal pregnancy, and the uE3 value was lower than normal pregnancy (*20*). Similarly, our data showed that the values of both AFP MoM and uE3 MoM decreased, while the value of β-hCG MoM increased. It is important to consider the serological screening test results, since clear deviations from the normal range also indicate an increased risk of chromosome abnormality. In our study, the screening risk of all 13 false negative cases were below the cut-off value (1:380); however, MoM values of some cases deviated significantly from the normal range. For example, the AFP MoM value of case number 4 was 0.52 (< 97.5%), the β-hCG MoM value of case number 5 was 3.68 (> 97.5%); and for case number 13, the AFP MoM value was 0.46 (< 97.5%) and the β-hCG MoM value was 3.68 (> 97.5%). Therefore, the MoM value of the serological markers should be taken into consideration when interpreting Down syndrome screening report, which could reduce the occurrence of false negative cases.

MoM values provide a simple way to compare the deviation of an individual from the overall population. When using software to calculate Down syndrome risk, the level of serological markers in pregnant women was correlated with their race, age, gestational age, weight, diabetes and smoking, and the correction of gestational age and weight is most significant (*21*). Several regression equations have been developed for risk estimate correction (*6,13,22*). In our hospital, gestational age was corrected by ultrasonographic measurements, and weight was corrected by the reciprocal correction equation (*22*).

Both the positive rate and false positive rate increase with the increase of detection rate, which may lead to unnecessary invasive prenatal diagnostic procedures. Generally, the positive rate and false positive of an optimal screening protocol should be below 10%. It is observed that protocol-3 and protocol-6 combining screening risk and different serological β-hCG MoM values (protocol-3: screening risk > 1:380 and β-hCG MoM value more than 2.213; protocol-6: screening risk > 1:380 and β-hCG MoM value > 2.603) increased the detection rate up to almost 80% while did not significantly increase the positive rate and false positive rate. It is of significant importance to incorporate ultrasound screening findings when interpreting serological examination results, so as to improve the detection rate of Down syndrome. As shown among the 13 false negative cases (Table 6), 6 cases showed abnormal soft indexes of ultrasound, including NF thickened, nasal bone hypoplasia, cardiac abnormalities, and head edema.

With the advancement of non-invasive prenatal testing technology, more and more pregnant women have chosen non-invasive prenatal testing (NIPT) to screen for fetal chromosome abnormalities (*23*). NIPT has the advantage of high accuracy and noninvasiveness (*23*). An increasing amount of data has shown that NIPT can be used in medium risk groups as stated by the International Society for Prenatal Diagnosis (*24*). In economically developed regions, for pregnant women with critical Down syndrome screening risk or abnormal serological indicator MoM values, using NIPT is advantageous. But in less developed regions, traditional Down syndrome screening remains the main method for chromosome abnormality screening. Therefore, choosing an appropriate screening protocol is particularly important. The detection rate of our protocol-3 and protocol-6 was close to 80%, in combination with prenatal ultrasound screening, and can detect most Down syndrome pregnancies.

As a routine method of prenatal examination in China, the Down syndrome triple serological screening test has the advantages of efficiency, economy and convenience. The main purpose of this study was to incorporate different MoM values of serological markers into the existing Down syndrome screening test, so as to increase the efficiency of Down syndrome screening. Our study with a relatively large sample size utilizing data from the last decade in our hospital showed a similar positive rate, detection rate and false positive rate to reports from other countries and regions (*25,26*). Protocols (protocol-3 and protocol-6) which combined serum β-hCG MoM value > 95% (2.213) or > 97.5% (2.603) with the traditional screening risk showed high detection rate, low positive rate and false positive rate, and could increase the efficiency. However, the results need to be further evaluated in a validation study. In addition, the overall incidence rate of Down syndrome was lower in our dataset, which may be related to a pre-selection of pregnant women in our hospital.

In conclusion, the efficiency of combining screening risk with serological marker MoM values to screen for Down syndrome needs to be prospectively evaluated in different centers with large sample sizes. Selection of an optimal screening protocol should be based on the population characteristics of each center.

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References

1. Stoll C, Dott B, Alembik Y, Roth MP. Associated congenital anomalies among cases with Down syndrome. Eur J Med Genet. 2015; 58:674-80.
2. V Plaiasu. Down Syndrome – Genetics and Cardiogenetics. Maedica (Buchar). 2017; 12:208-213.
3. Spencer, K. Screening for Down syndrome. Scand J Clin Lab Invest Suppl. 2014; 244:41-7.
4. Palomaki GE, Eklund EE, Neveux LM, Lambert Messerlian GM. Evaluating first trimester maternal serum screening combinations for Down syndrome suitable for use with reflexive secondary screening via sequencing of cell free DNA: High detection with low rates of invasive procedures. Prenat Diagn. 2015; 35:789-96.
5. Jiang T, Sun YJ, Xu QJ, Sun Y, Zhang XJ, Cao L, Z W, Zhang J, Huang ML, Chen CH, Lin YS, Xu ZF. Results of second-trimester prenatal screening using two serum markers for Down's syndrome in 60 931 pregnant women. Chin J Perinat Med. 2011; 14:74-77. (Article in Chinese)
6. Malone, FD, Canick JA, Ball RH, *et al*. First-trimester or second-trimester screening, or both, for Down's syndrome. N Engl J Med. 2005; 353:2001-2011.
7. Alldred SK, Deeks JJ, Guo B, Neilson JP, Alfirevic Z. Second trimester serum tests for Down's Syndrome screening. Cochrane Database Syst Rev. 2012; 6:CD009925.
8. Alldred SK, Takwoingi Y, Guo B, Pennant M, Deeks JJ, Neilson JP, Alfirevic Z. First and second trimester serum tests with and without first trimester ultrasound tests for Down's syndrome screening. Cochrane Database Syst Rev. 2017; 3:CD012599.
9. Ananth CV, Wapner RJ, Ananth S, D'Alton ME, Vintzileos AM. First-Trimester and Second-Trimester Maternal Serum Biomarkers as Predictors of Placental Abruption. Obstet Gynecol. 2017; 129:465-472.
10. Reynolds TM, Vranken G, Van Nueten J. Weight correction of MoM values: Which method? J Clin Pathol. 2006; 59:753-758.
11. Zhang Y, Hu J, Qiu C. Clinical application of TCSoft Down's syndrome screening software for prenatal screening in middle period pregnant women. Lab Med Clin. 2013; 10 (Suppl I):55-58. (Article in Chinese)
12. Wald NJ. Prenatal screening for open neural tube defects and Down syndrome: Three decades of progress. Prenat Diagn. 2010; 30:619-621.
13. Jiang Y, Liu J, Liu S, Hao N, Zhou J, Qi QZ, Zhou XY, Bian XM. The Application Instance of Monitoring and Audit of Median values of Mom in Prenatal Screening of Down's Syndrome. Journal of Practical Obstetrics and Gynecology. 2014; 30:103-107. (Article in Chinese)
14. Akolekar R, Beta J, Picciarelli G, Ogilvie C, D'Antonio F. Procedure-related risk of miscarriage following amniocentesis and chorionic villus sampling: A systematic review and meta-analysis. Ultrasound Obstet Gynecol. 2015;45:16-26.
15. Benn P, Borell A, Chiu R, *et al*. Position statement from the Aneuploidy Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn. 2013; 33:622-9.
16. Agarwal Gupta N, Kabra M. Diagnosis and management of Down syndrome. Indian J Pediatr. 2014; 81:560-7.
17. Dong Y, Yan X. Significance and feasibility of combined serological and ultrasound screening for early diagnosis of fetal Down's syndrome. Chinese Journal of Practical Gynecology and Obstetrics. 2010; 12:895-898. (Article in Chinese)
18. Gomes MS, Carlos-Alves M, Trocado V, Arteiro D, Pinheiro P. Prediction of adverse pregnancy outcomes by extreme values of first trimester screening markers. Obstet Med. 2017; 10:132-137.
19. Merkatz IR, Nitowsky HM, Macri JN, Johnson WE. An association between low maternal serum alpha-fetoprotein and fetal chromosomal abnormalities. Am J Obstet Gynecol. 1984; 148:886-94.
20. Wald NJ, Kennard A, Densem JW, Cuckle HS, Chard T, Butler L. Antenatal maternal serum screening for Down's syndrome: Results of a demonstration project. BMJ. 1992; 305:391-394.
21. Huang T, Meschino WS, Okun N, Dennis A, Hoffman B, Lepage N, Rashid S, Aul R, Farrell SA. The impact of maternal weight discrepancies on prenatal screening results for Down syndrome. Prenat Diagn. 2013; 33:471-476.
22. Zhang B, Liu X. Influence of maternal weight on MoM and screening performance in second trimester prenatal triple marker screening. Laboratory Medicine. 2014; 29:1101-1106. (Article in Chinese)
23. Du Y, Lin J, Lan L, Dong Y, Zhu J, Jiang W, Pan X, Lu Y, Li D, Wang L. Detection of chromosome abnormalities using current noninvasive prenatal testing: A multi-center comparative study. Biosci Trends. 2018; 12:317-324.
24. Benn P, Borrell A, Chiu RW, *et al.* Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn. 2015; 35:725-734.
25. Kazerouni NN, Currier B, Malm L, Riggle S, Hodgkinson C, Smith S, Tempelis C, Lorey F, Davis A, Jelliffe-Pawlowski L, Walton-Haynes L, Roberson M. Triple-marker prenatal screening program for chromosomal defects. Obstet Gynecol. 2009; 114:50-58.
26. Abou-Youssef HS, Kamal MM, Mehaney DA. Triple test screening for Down syndrome: An Egyptian-tailored study. PLoS One. 2014; 9:e110370.

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