Optimization of regional median equations of prenatal screening markers for trisomy 21 in a Chinese population

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Abstract
To establish gestational age-specific and body weight-specific mid-trimester normal median equations for the prenatal serum markers α-fetoprotein (AFP), free β subunit human chorionic gonadotropin (βhCG), and unconjugated oestriol (uE3) for a Chinese population; to compare and replace the median equations built in LifeCycle software; to evaluate the effect of equations used for gestation correction on estimating risk in Down’s syndrome, Edward’s syndrome, and neural tube defect (NTD).

A total of 353,065 cases of prenatal screening data of pregnant women were screened by 13 prenatal screening institutions in China. The local median equations of each institution and the large data were fitted by the least square regression, and then the difference was compared between large data equations and local median equations. The applicability of the localized median equations was evaluated by the determination coefficient. Based on the established median equations, multiples of median (MoM) of each values were calculated and compared with the latest Down’s syndrome quality assurance support service (DQASS).

There is no significant difference between the local median equations of each institution and the large sample median equations, which are various from LifeCycle built-in median equations. Besides, the determination coefficient of localized median equations are >0.99. 97.0% MoM medians obtained by using local median equations are consistent with latest standard of DQASS.

The median established by large sample data represents the median level of a Chinese population, and can be used to replace the software built-in median equations to achieve better screening results.

Abbreviations: AFP = markers α-fetoprotein, DQASS = Down’s syndrome quality assurance support service, βhCG = free β subunit human chorionic gonadotropin, FASP = Fetal Anomaly Screening Programme, MoM = multiples of median, NTD = neural tube defect, R² = determination coefficient, uE3 = unconjugated oestriol.

Keywords: least square regression, median equation, MoM, prenatal screening

1. Introduction
Risk estimation in second-trimester prenatal screening for chromosomal anomalies, such as Down’s syndrome (trisomy 21) or Edward’s syndrome (trisomy 18) is often based on the triple markers test.[1] With this test, 3 maternal serum markers—namely, alpha-fetoprotein (AFP), free beta subunit of human chorionic gonadotropin (βhCG), and unconjugated oestriol (uE3) are measured to estimate the pregnancies risks. For example, low concentrations of AFP together with increased βhCG and low concentrations of uE3 are frequently found in women carrying Down syndrome fetuses.[2] A separate screening protocol identifies some cases of trisomy 18, which is associated with low concentrations of AFP, βhCG, and uE3.[3] Therefore, effective and accurate screening relies upon the establishment of the median concentrations of AFP, βhCG, and uE3 in pregnant women. The median equation plays a crucial role in the risk assessment of prenatal screening.[4] Inaccuracy in equation settings can directly lead to inaccurate screening risk values, leading to the low detection, and high false positive rate of birth defects such as Down’s syndrome.

Maternal serum analytes, AFP, βhCG, and uE3 values have been shown to be influenced by gestation, maternal weight,[5] race, and ethnicity,[6-8] the presence of certain conditions like insulin-dependent diabetes,[6] and smoking.[9] As the levels of the 3 markers change with gestational age and body weight, the results of these markers are expressed as multiples-of-median (MoM). These MoM values are compared with parametric population statistics and combined with a prior risk based on maternal age to calculate the likelihood of chromosomal aneuploidy and neural tube defects (NTD).[10] It is suggested to keep median MoM levels close to the target by making changes to remove biases which will continue.

The built-in median equations for the risk assessment software are based on Caucasian population, which affects the accuracy of screening results in China due to the intrinsic difference between
Chinese and Caucasian population. To study and optimize MoM specific for Chinese population, adequate screening sample size and institutions with adequate annual screening sizes are important. In this study, we analyze screening data from 13 institutions in China in order to evaluate localization of the median equation in this population, improving accuracy of prenatal screening for chromosomal anomalies.

2. Material and methods

2.1. Research subjects

This study was carried out at 13 screening laboratories in China as shown in Figure 1. All pregnancies are singleton, nondiabetic, and nonsmoker pregnancies with normal outcomes only. Data was collected at 98 to 146 days of gestation for test referrals to our laboratories from January to December, 2015. Prenatal screening involved 353,065 subjects in total. All pregnant women volunteering to participate in prenatal screening were counseled about triple testing and signed the informed consent. This study was approved by the Hospital Ethics Review Board and the approval number is Batch 2014, No. 042.

All the basic information of pregnant women including the date of birth, the last menstruation, body weight, date of prenatal screening, the number of fetuses, smoke history, type I diabetes mellitus history, and pregnancy history were collected.

2.2. Prenatal screening

Maternal plasma samples (2–3 mL) were collected from all pregnant women. The concentration of AFP, free βhCG, and uE3 were detected by time-resolved fluoroimmunoassay. AFP and free βhCG results were expressed in IU/mL and uE3 in nmol/L.

2.3. Statistical analysis

Data derived from 353,065 serum samples was examined in this study. Data were organized by using Microsoft Excel (Microsoft Corporation) and the gestational age and body weight of all pregnant women were analyzed by SPSS 17.0 software (SPSS Inc.). Median values for second trimester maternal serum markers were established as described by Vranken et al.[1] Least squares regression was used to establish median equations of AFP, free ßhCG, and uE3 by R language against gestational age (in days) and maternal weight and the fitting effect of median equations were judged by the corrected determination coefficient ($R^2$). Based on the established median equations, MoM of each values were calculated and compared with the latest DQASS
standards. Moreover, MoM determined the risk of carrying a fetus with trisomy 21, trisomy 18, and NTD by Wallace LifeCycle Elipse risk calculation engine (Perkin Elmer).

3. Results

3.1. Data acquisitions

This study employed data from 353,065 pregnant women in the second trimester, excluding multiple pregnancy, type I diabetes, smoking, poor pregnancy history, and abnormal pregnancy outcomes. As shown in Table 1, Fujian Provincial Hospital (FPH), Fuzhou General Hospital Affiliated to Nanjing Military Region (FGH) and Nanping City First Hospital (NCFH) had <5000 screening tests, while the other 10 prenatal screening institutions had annual screening capacities of more than 20,000. The average age of pregnant women at screening institutions was 27.36 years old with the average body weight of 54.33kg. The average gestational days were 118 to 124 days with small standard deviations among different institutes.

3.2. AFP, fβhCG, and uE3 median distribution with gestational age

The medians of 3 serum markers, AFP, fβhCG, and uE3, were calculated and the median distribution among all the screening institutions was analyzed with gestational days. The results are shown in Figure 1. Compared with other 11 prenatal screening institutions, there is a significant difference in all 3 types of median values from FGH and NCFH. This discrepancy may be related to small number (839 and 1287) of screening tests done in FGH and NCFH. Therefore, data from both screening agencies were excluded for subsequent fitting of the median equations.

3.3. Median equations of AFP with gestational age and body weight

As shown in Figure 2, we can see that the median equations fitted by AFP values from each screening institution have high consistency, which is different from the built-in median equations of LifeCycle risk assessment software. As can be seen from Table 2, $R^2$ of each

| Number | Hospital Name                                             | Screening quantity | Age | SD | Gestational age (day) | SD | Weight (kg) | SD |
|--------|-----------------------------------------------------------|--------------------|-----|----|-----------------------|----|-------------|----|
| 1      | Fujian Province Maternal and Child Health Hospital        | 35,936             | 27.55 | 3.78 | 124.78                 | 7.16 | 55.31       | 8.11 |
| 2      | Fujian Provincial Hospital                               | 4602               | 28.94 | 3.57 | 119.31                 | 7.66 | 55.11       | 8.03 |
| 3      | Fuzhou City First Hospital                              | 31,550             | 27.07 | 3.84 | 123.87                 | 8.08 | 54.67       | 8.38 |
| 4      | Longyan City First Hospital                             | 27,081             | 27.82 | 4.18 | 124.93                 | 8.01 | 53.79       | 7.93 |
| 5      | Fujian East Hospital                                     | 29,452             | 27.25 | 3.79 | 123.02                 | 7.79 | 54.17       | 7.73 |
| 6      | Fuzhou General Hospital Affiliated to Nanjing Military Region | 839               | 28.53 | 3.31 | 118.60                 | 6.96 | 54.37       | 7.47 |
| 7      | Nanping City First Hospital                             | 1287               | 27.61 | 3.46 | 121.47                 | 7.01 | 55.35       | 8.27 |
| 8      | Nanping City Maternal and Child Health Hospital          | 21,178             | 27.11 | 3.85 | 123.85                 | 7.70 | 54.67       | 8.30 |
| 9      | Putian City Maternal and Child Health Hospital           | 20,113             | 26.70 | 3.49 | 123.70                 | 8.26 | 55.02       | 8.58 |
| 10     | Quanzhou City Maternal and Child Health Hospital         | 67,540             | 27.19 | 3.75 | 123.40                 | 8.34 | 54.24       | 8.31 |
| 11     | Sanming City First Hospital                             | 22,657             | 27.15 | 3.77 | 123.52                 | 7.56 | 53.87       | 7.99 |
| 12     | Xiamen City Maternal and Child Health Hospital           | 49,023             | 28.00 | 3.69 | 122.07                 | 9.42 | 54.28       | 8.11 |
| 13     | Zhangzhou City Hospital                                 | 41,057             | 27.07 | 3.91 | 123.06                 | 8.49 | 53.49       | 8.23 |
| Total  |                                                          | 353,065            | 27.36 | 3.82 | 123.42                 | 8.27 | 54.33       | 8.19 |

SD = standard deviation.

The number refers to the name of the hospital.

**Table 1**

Descriptive statistics of 13 prenatal screening institutions in Fujian Province in 2015.

3.2. AFP, fβhCG, and uE3 median distribution with gestational age

The medians of 3 serum markers, AFP, fβhCG, and uE3, were calculated and the median distribution among all the screening institutions was analyzed with gestational days. The results are shown in Figure 1. Compared with other 11 prenatal screening institutions, there is a significant difference in all 3 types of median values from FGH and NCFH. This discrepancy may be related to small number (839 and 1287) of screening tests done in FGH and NCFH. Therefore, data from both screening agencies were excluded for subsequent fitting of the median equations.

3.3. Median equations of AFP with gestational age and body weight

As shown in Figure 2, we can see that the median equations fitted by AFP values from each screening institution have high consistency, which is different from the built-in median equations of LifeCycle risk assessment software. As can be seen from Table 2, $R^2$ of each.

![Figure 2. Median equations of AFP with gestational age and body weight from 11 prenatal screening institutions. AFP = markers α-fetoprotein.](image-url)
Table 2
Median equations and determination coefficient of AFP from 11 prenatal screening institutions.

| Institute number | Gestational days |\( R^2 \) | Weight |\( R^2 \) |
|------------------|-----------------|---------|--------|---------|
| 1                | \(10^{-0.894622+0.00043388×GD+0.00002087008×GD^2} \) | 0.982  | 0.313466×37.1942×(1/W) | 0.998 |
| 2                | \(10^{-0.0587615+0.0003072038×GD+0.0000205186×GD^2} \) | 0.980  | 0.2593×39.95W | 0.995 |
| 3                | \(10^{-0.961597+0.00070497×GD+0.0000205186×GD^2} \) | 0.990  | 0.295514×37.8106×(1/W) | 0.999 |
| 4                | \(10^{-1.22382+0.0000137819×GD+0.00000409531×GD^2} \) | 0.987  | 10\(^{0.435784-0.010492×W}×W+0.0000428915×W^2\) | 0.989 |
| 5                | \(10^{-0.0000809342+0.00150019×GD+0.0000154069×GD^2+0.000000477077×GD^3} \) | 0.983  | 10\(^{0.421852-0.0100677×W}×W+0.0000408318×W^2\) | 0.990 |
| 8                | \(10^{-0.751637+0.000536326×GD+0.0000140785×GD^2} \) | 0.991  | 0.295063×37.9086×(1/W) | 0.999 |
| 10               | 135.359-2.3898×GD+0.0313195×GD^2 | 0.982  | 10\(^{0.359625-0.00779606×W}×W+0.000216654×W^2\) | 0.966 |
| 11               | \(10^{-0.948799+0.00021626×GD+0.000034215×GD^2} \) | 0.986  | 10\(^{0.400394-0.00297678×W}×W+0.000335786×W^2\) | 0.982 |
| 12               | \(10^{-0.00004695×GD+0.000005211×GD^2} \) | 0.975  | 2.092-0.0277×W×W+0.000136×W^2 \) | 0.954 |
| 13               | \(10^{-0.061117+0.000189877×GD+0.0000305485×GD^2} \) | 0.993  | 0.296124×37.7159×(1/W) | 1.000 |
| All data         | \(10^{-0.880755+0.00039424×GD+0.0000215047×GD^2} \) | 0.998  | 0.277134×37.9569×(1/W) | 0.996 |

AFP = markers α-fetoprotein, GD = gestational day, \( R^2 \) = determination coefficient, W = weight.

The median equations from all data with gestational days and body weight are both 0.998. The details of the parametric equation are shown in Table 2.

3.4. Median equations of \( \beta hCG \) with gestational age and body weight

As shown in Table 3, \( R^2 \) for the median equations of the \( \beta hCG \) from each screening institution ranges from 0.904 to 1.000, indicating that the parametric equations among the screening institutions have some differences, which may be related to the fact that \( \beta hCG \) is not stable. As can be seen from Figure 3, the median equations for the \( \beta hCG \), which differ from the median equation built into the LifeCycle risk assessment software, fit for each screening agency. \( R^2 \) of the median equations from all data with gestational days and body weight are 0.998 and 0.994, respectively.

Table 3
Median equations and determination coefficient of \( \beta hCG \) from 11 prenatal screening institutions.

| Institute number | Gestational days |\( R^2 \) | Weight |\( R^2 \) |
|------------------|-----------------|---------|--------|---------|
| 1                | \(10^{-0.957219+0.1092598×GD+0.000025785×GD^2+0.00000410527×GD^3} \) | 0.988  | 0.1347695×46.5627×(1/W) | 0.997 |
| 2                | \(10^{(7.57826-0.931851×GD+0.00032912×GD^2} \) | 0.977  | 0.2337×41.71×(1/W) | 0.990 |
| 3                | \(853.575-18.0405×GD+0.129524×GD^2+0.00031265×GD^3 \) | 0.992  | 0.131563×46.7305×(1/W) | 0.997 |
| 4                | \(10^{-13.7231-0.250799×GD+0.00166622×GD^2+0.0000375784×GD^3} \) | 0.922  | 0.222361×40.9469×(1/W) | 0.994 |
| 5                | \(3945.97-113.411×GD+0.22955×GD^2) \) 0.0000569×G D^3 | 0.904  | 10\(^{0.385508-0.00768003×W}×W+0.0000921997×W^2\) | 0.927 |
| 8                | \(10^{(0.07185-0.0524908×GD+0.000160899×GD^2+0.0000153642×GD^3) \) | 0.978  | 0.10\(^{0.367382-0.00674852×W}×W) | 0.913 |
| 9                | \(10^{(4.65525-0.0494024×GD+0.0000135498×GD^3) \) | 0.990  | 10\(^{0.351395-0.006145302×W}×W\) | 0.924 |
| 10               | \(10^{-0.915404-0.0605612×GD^2+0.000216816×GD^2} \) | 0.995  | 0.1676208×44.3918×(1/W) | 1.000 |
| 11               | \(10^{-0.772-0.06286×GD+0.00002011×GD^2+0.0000040608×GD^3} \) | 0.978  | 10\(^{0.4287-0.009575×W+0.00002382×W^2}×W\) | 0.967 |
| 12               | \(10^{-0.597912+0.097639×GD+0.0000153642×GD^2+0.0000216816×GD^2+0.0000040608×GD^3} \) | 0.990  | 10\(^{0.347623-0.00645068×W}×W\) | 0.909 |
| 13               | \(10^{(8.18576-0.0705427×GD+0.0000235012×GD^2+0.0000216816×GD^2} \) | 0.983  | 10\(^{0.485017-0.0113532×W}×W+0.0000414899×W^2\) | 0.984 |
| All data         | \(10^{(5.91707-0.0658925×GD+0.0000216816×GD^2} \) | 0.998  | 0.1751×44.09×W | 0.994 |

\( \beta hCG \) = free α-subunit human chorionic gonadotropin, GD = gestational day, \( R^2 \) = determination coefficient, W = weight.
that there is a difference in the median equations from each screening agency. As shown in Figure 4, the median equations of uE3 concentration with gestational days have a significant difference from the built-in median equation of the LifeCycle, while the difference of the median equation of body weight is small. However, $R^2$ of the median equations from all data with gestational days and body weight are 1.000 and 0.993, respectively.

3.6. The evaluation of the application effect of median equation obtained from large sample data

Based on the median equation fitted by large sample data in Fujian Province, the MoM and MoM median of 3 kinds of serum marker in each screening institution were calculated. The detailed results are shown in Table 5. Except FGH and NCFH, the MoM median values of other 11 screening agencies range from 0.95 to 1.05, of which 97.0% (64/66) are in line with the latest MoM prescribed by DQASS. This shows that the equations obtained from the big data of each marker can reflect the median level of each institution and can be used to replace the built-in equations of LifeCycle software.

4. Discussion

The NHS Fetal Anomaly Screening Programme (FASP) has published a standard for quality assurance of MoM in Down’s syndrome screening. \[1\] Down’s syndrome quality assurance support service (DQASS) is based on an analysis of a large

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**Table 4**

Median equations and determination coefficient of uE3 from 11 prenatal screening institutions.

| Institute Number | uE3 Median equations | Gestational days | Weight | $R^2$ |
|------------------|----------------------|------------------|--------|-------|
| 1                | 74.35603–1.97425+0.0170385*GD+0.00004459901*GD^3 | 0.983 | 0.733505+14.3959*(1/W) | 0.897 |
| 2                | 10^(-3.99645+0.0643745*GD+0.000204894*GD^2) | 0.942 | 0.6446+19.33*(1/W) | 0.953 |
| 3                | 10^(-3.37854+0.0542165*GD+0.000164855*GD^2) | 0.977 | 10^(-0.1897920.00434739*W+0.0000156409*W^2) | 0.981 |
| 4                | 10^(-7.88229+0.145319*GD-0.000000656*GD^2+0.0000202273*GD^3) | 0.991 | 10^(-0.2063650.00596781*W+0.0000223988*W^2) | 0.998 |
| 5                | 10^(-7.50414+0.153433*GD-0.000960693*GD^2+0.0000213851*GD^3) | 0.975 | 10^(-0.19733–0.00454773*W+0.0000162564*W^2) | 0.962 |
| 8                | 10^(-2.34085–0.0860606*GD+0.0000767917*GD^2+0.00000271831*GD^3) | 0.988 | 10^(-0.135624–0.00249892*W) | 0.843 |
| 9                | 1.70763–0.07035301*GD+0.000111633 *GD^2 | 0.977 | 10^(-0.141407–0.00259056*W) | 0.832 |
| 10               | 10^(-2.94925+0.0478231*GD-0.000140917*GD^2) | 0.901 | 10^(-0.1715340.00390293*W+0.0000132087*W^2) | 0.981 |
| 12               | 10^(-3.8305+0.145319*GD-0.000000656*GD^2+0.0000202273*GD^3) | 0.994 | 0.6765+16.97W | 0.979 |
| 13               | 10^(-3.14886+0.0507376*GD-0.000149269*GD^2) | 0.982 | 1.36021–0.006722499*W | 0.898 |
| All data         | 10^(-2.98763+0.0480736*GD-0.000140214*GD^2) | 1.000 | 1.478–0.01171*W+0.00005254*W^2 | 0.993 |

GD = gestational day, $R^2$ = determination coefficient, uE3 = unconjugated oestriol, W = weight.
number of UK data and proposes a universal standard for prenatal screening for MoM median analysis, with the whole world recognition. The recently updated standard specifies that “The median of the MoM values used in risk calculation, for any subpopulation defined by time period, gestational age, maternal weight, smoking status and ethnicity should lie within 5% of the target value of 1, that is, the median adjusted MoM value should lie between 0.95 and 1.05.” To achieve this standard, it is recommended that laboratories monitor a whole range of different parameters to ensure that when median recalculation is necessary this will be carried out with a minimum of delay, thereby ensuring compliance with the standard.

At present, maternal serological screening is still the mainstream to monitor and prevent 21 trisomy syndrome, 18 trisomy syndrome, and NTD in China. To improve the quality of screening and to establish effective quality control standards, accuracy for prenatal screening are hot topics prenatal screening experts and the major screening agencies in China.[12,13] A number of studies have shown that the median levels of 3 serum markers, AFP, free β-hCG, and uE3, have ethnic and regional differences. Moreover, the main screening institutions in China use prenatal screening risk assessment software developed abroad, such as LifeCycle and 2T. Therefore, the localization of the median equations for prenatal screening risk assessment software is of particular importance.

This study included 353,065 cases of normal pregnant women in 13 screening institutions in Fujian Province. The sample size was much larger than the previous median equation studies. Ten screening institutions screened have more than 20,000 cases, which better represents the normal population level in all regions of Fujian Province, China. Meanwhile, all the screening data of AFP, free βhCG, and uE3 concentration were measured by time-resolved fluorescence immunoassay and the maternal risk probability were calculated by LifeCycle software. All the above information is to ensure that the representation, accuracy, and reliability of the screening data in Fujian Province, China.

From Figures 2 to 4, it is shown that the median equation of gestational age and weight fitted by the large sample data have

Table 5
Retrospectively calculated median MoM from large sample median equations.

| Institution | AFP MoM median | free β-hCG MoM median | uE3 MoM median |
|-------------|----------------|-----------------------|----------------|
|             | GD  | Weight | GD  | Weight | GD  | Weight |
| 1           | 1.014 | 1.030 | 1.010 | 1.027 | 0.978 | 0.985 |
| 2           | 0.996 | 1.000 | 1.027 | 1.050 | 1.022 | 1.026 |
| 3           | 0.986 | 0.992 | 1.048 | 1.055 | 0.957 | 0.960 |
| 4           | 0.981 | 0.976 | 1.041 | 1.029 | 1.006 | 1.005 |
| 5           | 1.027 | 1.027 | 1.038 | 1.039 | 1.010 | 1.010 |
| 6           | 0.996 | 1.001 | 1.067 | 1.062 | 0.960 | 0.956 |
| 7           | 1.022 | 1.043 | 1.069 | 1.105 | 0.946 | 0.953 |
| 8           | 0.996 | 1.004 | 1.020 | 1.029 | 0.989 | 0.991 |
| 9           | 1.023 | 1.032 | 1.024 | 1.040 | 1.018 | 1.025 |
| 10          | 1.001 | 1.000 | 0.967 | 0.969 | 0.987 | 0.987 |
| 11          | 1.007 | 1.005 | 0.983 | 0.977 | 1.016 | 1.014 |
| 12          | 0.996 | 0.999 | 0.987 | 0.989 | 1.015 | 1.015 |
| 13          | 0.982 | 0.972 | 0.954 | 0.944 | 1.036 | 1.033 |

GD = gestational day, MoM = multiples of median.
high consistency with the one fitted in each screening institution, but the curve of the median equations from built-in LifeCycle deviates significantly from the localized equation. As shown in Tables 2–4, under the condition of large sample data, the determination coefficients of the median equations of each marker are all >0.99. The MoM median of each serum marker is calculated by the localized median equation, among which, 97.0% MoM medians (shown in Table 5) meet the latest DQASS standards.[11] These results indicate that the median equations of screening agencies are very well fitted. The larger the sample data, the better the fitting effect of the median equations and the more representative the median equations are.

Several studies have found that the median equations after localization for prenatal screening risk assessment software such as LifeCycle can increase the detection rate of trisomy 21 and reduce the false-positive rate, with overall better results than the median equations without localization, which can effectively prevent and control the birth of children with birth defects such as trisomy 21.[1,13] At present, many regions in China have achieved the median equation localization. However, due to the limited amount of prenatal screening data and the factors that affect the stability of the experiment, the localization of median equations in many prenatal screening institutions still cannot be achieved. In view of this, the median equations fitted in this study can be used in Fujian province, China to replace the median equations built-in the software, and can provide a reference and scientific basis for the regions where the median equation is not localized yet.

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