Automated Trisomy 21 Assessment Based on Maternal Serum Markers Using Trivariate Lognormal Distribution

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Abstract: - Trisomy 21 is the most frequent types of chromosomal abnormalities. Generally, current methods for trisomy risk assessment are divided into two techniques, which are invasive and non-invasive methods. Invasive methods are including amniocentesis, chorionic villus sampling (CVS), or percutaneous umbilical cord blood sampling (PUBS), but its drawbacks are expensive, time consuming and having risk of miscarriage, where else non-invasive methods are counting on ultrasound marker and maternal serum markers screening. Nevertheless, single evaluations on ultrasound markers itself are always not enough for risk assessment in terms of its accuracy, reliability and consistency. So, we proposed a new mathematical algorithm which combines three maternal serum markers using trivariate lognormal distribution to calculate automatically the probability or likelihood that a woman has an affected pregnancy or not. The developed algorithm was implemented into graphical user interface to act as computer aided e-diagnostic system. We have compared the results with published finding and found it is almost equally accurate.

Key-Words: - Trisomy 21, maternal serum marker, maternal health data, trivariate distribution, bivariate distribution, chromosomal abnormalities, multivariate distribution

1 Introduction

A very common abnormality such as trisomy 21, 18, and 13 results from having an extra copy of chromosome respectively instead of two normal copies [1]. Trisomy 21 or Down's syndrome perhaps is one of the most frequent congenital causes of severe mental retardation with an incidence at birth 1.3 per 1000 [2]. In general, chromosomal abnormalities can be detected through existing techniques including genetic testing, maternal serum markers and ultrasound markers prenatal screening. Genetic testing is categorized into invasive method such as amniocentesis, CVS and PUBS. Due to its limitations in term of time usage, cost and potential miscarriage risk, it is always been considered as non-preferred technique during premature fetal screening and only been regarded for confirmatory testing at the last stage of clinical abnormalities screening.

Besides, ultrasound markers prenatal screen offer promising non-invasive techniques for fetal abnormalities detection, such as nuchal translucency (NT), nasal bone, long bone biometry and ductus venous [4]. An increased NT thickness that more than 2.5mm in between 10 and 13 weeks plus six days has also been associated with an increased risk of congenital heart and genetic syndrome [5] [6] [7]. However, a single evaluation on ultrasound markers to assess the likelihood of trisomy 21 is not sufficient and its accuracy is not satisfied and reliable. Therefore, studies of maternal serum markers appear as important method to further improve the accuracy of previous ultrasound measuring. Clarisse B. et al. [8] had proved a benefit in combining ultrasound markers and biochemical markers in the first or second trimester for Down's syndrome risk screening. Maternal serum markers serve as one of the laboratory screenings to track down the occurrence of trisomy 21 combining with maternal data [2] [3]. It enables the improvement of effectiveness of antenatal screening for Down's syndrome by measuring concentration of particular biochemical markers [2].

Based on previous literatures, maternal serum markers are defined as a hormone or protein found in maternal blood that can be served as a sign of abnormality. The most common of these markers being alpha fetoprotein (AFP), pregnancy associated plasma proteins A (PAPP-A), unconjugated oestriol (uE3), free ß-human chorionic gonadotrophin (free ß-hCG) and inhibin A (DIA). It has been recognized that the chromosomally abnormal pregnancy is associated with the abnormal level of maternal serum markers. Both AFP and UE3 are produced by fetus while DIA, PAPP-A and free ß-hCG are produced by placental trophoblast during pregnancy [9].

In the first trimester, the PAPP-A level is, on average, low in Down's syndrome pregnancies (about

half that of unaffected pregnancies) [10]. In the second trimester AFP and uE3 levels are, on average, low (about three-quarters that of unaffected pregnancies) and inhibin- A and free ß-hCG levels are, on average, high (about double that of unaffected pregnancies). Also, K. O. Kagan et al. [3] had demonstrated that the maternal serum markers screening for calculation of accurate patient-specific risks for trisomy 21 is essential to take into account gestation age, maternal weight, ethnicity, smoking status and method of conception. They suggested the performance of the biochemical test is substantially better at 11 to 12 weeks than at 13 weeks.

Keeping the facts above, we present an automated computerized algorithm includes all the mathematical equations and formulas derivations to estimate the risk based on maternal serum markers and maternal data. For women with first trimester pregnancy, we have only used two domain maternal serum markers including PAPP-A and free ß-hCG to calculate their trisomy risk through bivariate distribution. Where else for pregnant women with second trimester of gestation, we have made used the combination of three maternal serum markers derived from trivariate algorithm, which are uE3, AFP and free ß-hCG. It will be more advantages of using more than two markers together in antenatal screening. The combinations of marker analysis give significantly more information than is given by any single marker alone, or by the group of markers when used sequentially [14]. Fig.1 displays each important step of developed software for risk calculation.

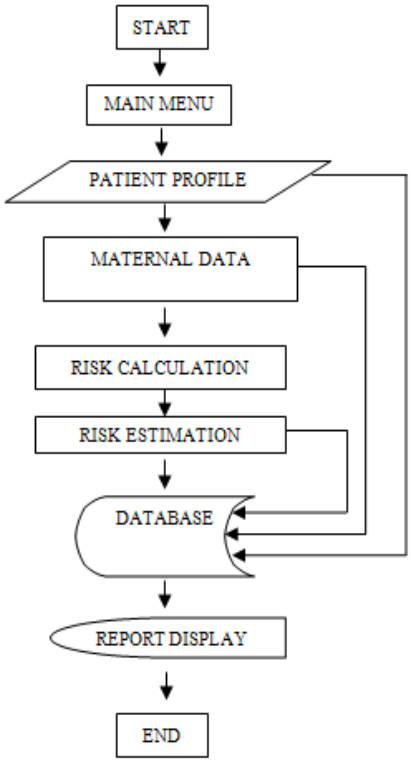


Fig. 1 Flowchart of the developed software.

In the following section 2, we describe the material and methods used to calculate the risk of trisomy 21. The results of present method are shown in Section 3, and finally we draw some conclusion in Section 4.

2 Material and Methods

In this section, we describe the parameters of maternal data including biochemical markers concentration in MoM, effect of maternal age and previous affected pregnancy for risk screening. It follows by mathematical formula derivations and risk calculation. In present studies, we have implemented the MoM values of PAPP-A, AFP, free ß-hCG and uE3 as the combination markers in multivariate calculation to produce the likelihood ratios. For the computation of MoM values, the women's measured serum value for each individual serum markers are divided by the expected median value found in women with normal pregnancies at the same gestation age. The MoM value of each marker can be converted into the likelihood ratio using log normal distribution of normal and affected pregnancy. In facts, the likelihood ratio was the height of the Gaussian distribution for the Down's syndrome pregnancies divided by the height of the Gaussian distribution for the unaffected pregnancies at the particular values of the variables concerned. It indicates the probability that a woman has an affected pregnancy or not. It is an efficient means of deriving information relating to a woman's risk of carrying an affected child.

2.1 Characteristics of Maternal Data

2.1.1 Concentration of Biochemical Markers

The concentration of the biochemical markers vary with the gestation age which unable valid comparisons to be made between concentrations at different stages of pregnancy. Hence, in order to take into account this variation effect, the level of the biochemical markers are expressed as a multiple of medians (MoM), in other words, the value of marker observed is divided by the expected median marker in unaffected pregnancy.

For example, if the level of free β-hCG is 2 MoM, it means that the concentration of free β-hCG is two times higher than the median concentration in normal pregnancy. The MoM distributions of each marker in normal and affected pregnancy usually follow the Gaussian distribution when the MoM is log transformed [2]. Fig. 2 illustrates example of the Gaussian (normal distribution) of free β-hCG in unaffected and Down syndrome pregnancy. The free β-hCG (MoM) is generally considered to be a better marker in the first trimester screening although it is less stable in whole blood specimens and must be separated within 5 hours of

collection to run an accurate essay [9]. Also, it is the only one maternal serum marker can be used in both the first and second trimester of pregnancy for risk assessment.

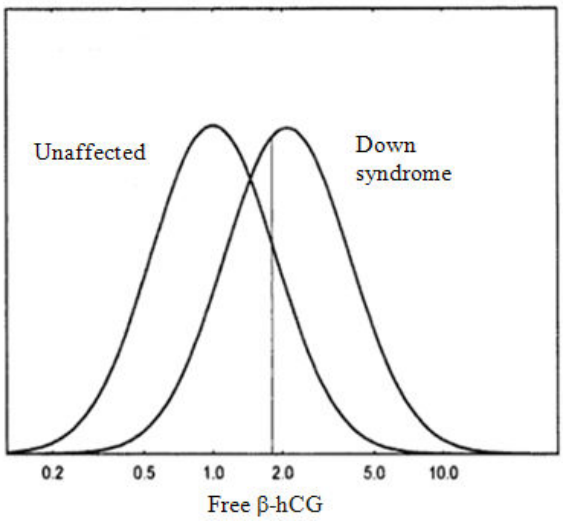


Fig. 2 The Probability Density Distribution of free β-hCG (MoM) in unaffected and Down syndrome pregnancy.

Except of the serum marker free β-hCG, PAPP-A is also one of the best maternal serum markers used in the first trimester pregnancy to detect chromosomal abnormalities. Fig. 3 illustrates example of the Gaussian (normal distribution) of PAPP-A in unaffected and Down syndrome pregnancy. PAPP-A is not only present in pregnant women but it also can be found in non-pregnant women and men as well.

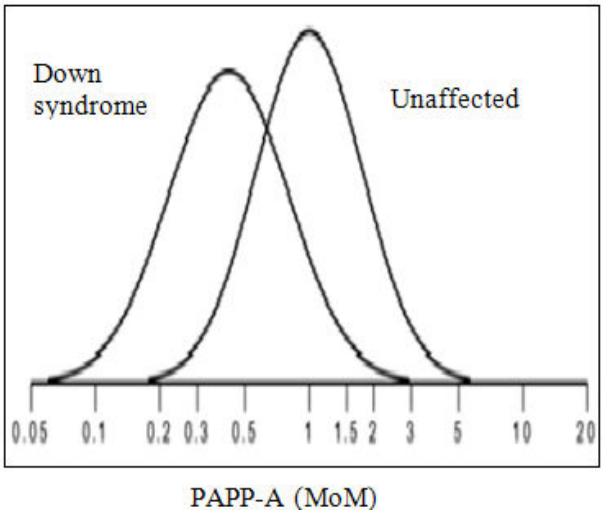


Fig. 3 The Probability Density Distribution of PAPP-A (MoM) in unaffected and Down syndrome pregnancy.

Alpha fetoprotein (AFP) also appears as a useful serum marker for chromosomal abnormalities assessment especially in second trimester pregnancy. It was found that the concentration of AFP in maternal blood tend to be low in maternal serum of trisomy pregnancy. This may due to the reduction in the transfer of this protein though the placenta into the maternal circulation in fetal chromosomal abnormalities. Fig. 4 illustrates example of the Gaussian (normal distribution) of AFP in unaffected and Down syndrome pregnancy.

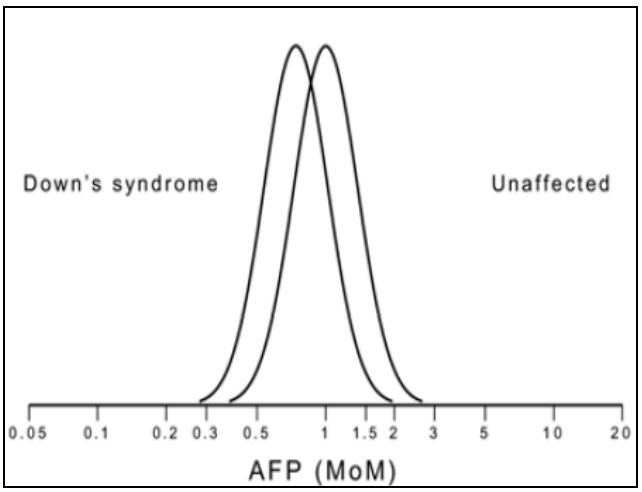


Fig. 4 The Probability Density Distribution of AFP (MoM) in unaffected and Down's syndrome pregnancy

Last but not least, the serum marker uE3 has been widely used during the second trimester as the additional combination markers for trisomy risk calculation. The concentration of uE3 rises rapidly especially during the later first trimester and early second trimester of pregnancy, where lower than normal levels of estriol may also indicate that a woman is at high risk for having baby with Down syndrome. Fig. 5 illustrates example of the Gaussian (normal distribution) of uE3 in unaffected and Down syndrome pregnancy.

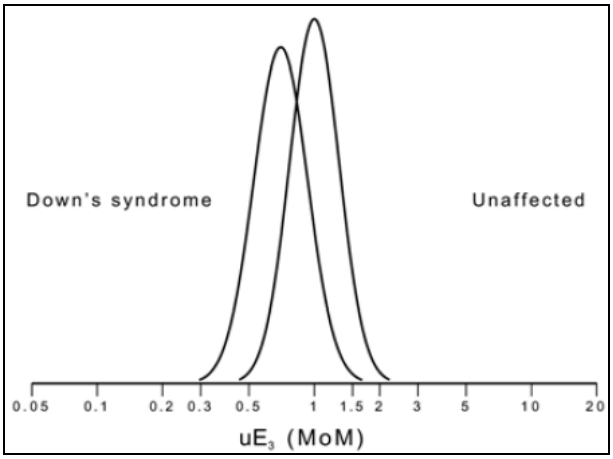


Fig. 5 The Probability Density Distribution of uE3 (MoM) in unaffected and Down syndrome pregnancy.

2.1.2 Effect of Maternal Age

Maternal age is the best known risk factor for trisomy 21 and other chromosomal abnormalities since 1980. The

reasons why the age of the mother increases the risk for chromosomal abnormalities are still unknown currently. However, one of idea that predicted by scientists is that older eggs are more prone to nondisjunction which in turn leads to the occurrence of trisomy 21, 18 and 13. For example, female eggs ovulated at age 40 have been in meiosis I for more than 40 years. During this time, events in the cell or environmental agents might damage the egg, making nondisjunction more likely.

According to P. Soergel et al. [11], first trimester screening using maternal age combining with maternal serum markers is highly effective for the detection of trisomy 21 and it is associated with a sensitivity of about 90% for 5% false-positive patients.

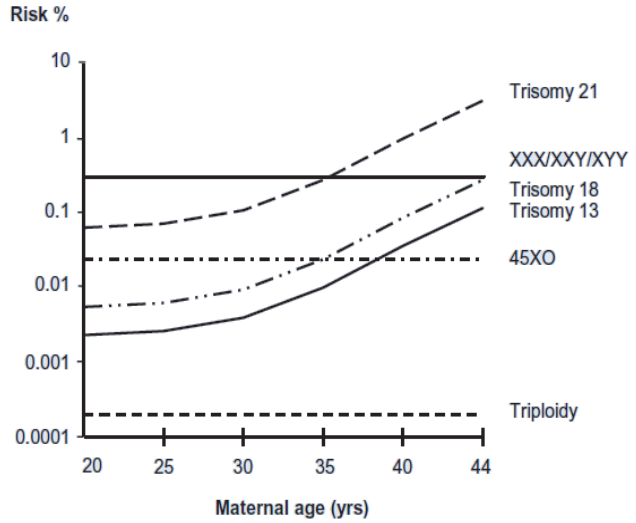


Fig. 6 Maternal age-related Risk for Chromosomes Abnormalities

Also, the risk of Down syndrome and some other chromosomal abnormalities in an unborn child is known to increase with the age of the mother and it is this knowledge which forms the basis for selection of pregnant women for further investigation [12]. Fig. 6 shows the example of maternal age-related risk for chromosomes abnormalities. It can be observed that the increase of maternal age will have positive exponential risk increment of trisomy 21.

2.1.3 Effect of Previous Affected Pregnancy

Normally, couples who have one child with trisomies have a slightly increased risk of having a second child with trisomies. The recurrent risk of trisomies increase in current pregnancy because some couple with a previously affected pregnancy have parental mosaicism or a genetic defect that interferes with the normal process of disjunction [13]. In woman who had a previous pregnancy with trisomies, the risk of recurrent in the subsequent pregnancy is 0.75% higher than maternal and gestational age-related risk for trisomies at the time of testing.

2.2 Mathematical Bivariate and Trivariate Derivations

The relevant likelihood ratio was derived from the multivariate Gaussian frequency distributions, since we proposed to use two and three maternal serum markers in our developed software for first and second trimester of pregnancy respectively, the appropriate bivariate and trivariate Gaussian distribution need to be derived. Multivariate distribution has been widely used in medical image data analysis and statistical studies [15-18]. Equation 1 below shows the general equation of multivariate normal distribution,

$$f(x) = \frac{1}{2\pi^{\frac{\rho}{2}}\left|\sum\right|^{\frac{1}{2}}} e^{\frac{-(x-\mu)\sum^{-1}(x-\mu)}{2}} \quad (1)$$

Where, $f(x)$ = probability of the (MoM) values for the combination markers; μ = transformed population mean for unaffected or affected pregnancies; ρ = correlation coefficient between transformed x for unaffected or affected pregnancies; x = transformed sample value, analyst x; σ = standard deviation of the transformed population for unaffected or affected pregnancies.

If two variables were used, for example, in our cases, PAPP-A and free ß-hCG during first trimester of pregnancy screening, the value ρ will be 2 and its distribution function is set out as follows,

$$\mu = \begin{bmatrix} \mu_1 \\ \mu_2 \end{bmatrix} \quad (2)$$

and the covariance matrix is,

$$\Sigma = \begin{bmatrix} \sigma_{11}^2 & \sigma_{12}^2 \\ \sigma_{21}^2 & \sigma_{22}^2 \end{bmatrix} \quad (3)$$

Then, the determinant of the covariance matrix can be expressed as follows,

$$Det(m_1) = \sigma_{11}^2\sigma_{22}^2 - \sigma_{12}^4 \quad (4)$$

Take note that the term σ_{12} and σ_{21} are always refer to the same serum markers, as shown in Equation 4 above. Next, by applying equation 4, the inverse covariance matrix and absolute covariance matrix can be formulated as follows,

$$\Sigma^{-1} = \frac{1}{\sigma_{11}^2\sigma_{22}^2 - \sigma_{12}^4}\begin{bmatrix} \sigma_{22}^2 & -\sigma_{12}^2 \\ -\sigma_{21}^2 & \sigma_{11}^2 \end{bmatrix} \quad (5)$$

$$\left|\Sigma^{\frac{1}{2}}\right| = \sqrt{Det(m)} = \sigma_{11}\sigma_{22}\sqrt{(1-\rho_{12}^2)} \quad (6)$$

Based on the previous established relationship, as shown in equation 7, the final bivariate function can be expressed based on equation 1 and simplified into equation 8 below,

$$\sigma_{12}^2 = \rho_{12}\sigma_{11}\sigma_{22} \quad (7)$$

$$f(x,y) = \frac{1}{2\pi\sigma_x\sigma_y\sqrt{1-\rho^2}}e^{-\frac{1}{2}\frac{1}{(1-\rho^2)}\{B\}} \quad (8)$$

which B denotes as follows,

$$\{B\} = \left(\frac{x-\mu_x}{\sigma_x}\right)^2 + \left(\frac{y-\mu_y}{\sigma_y}\right)^2 - \left(2\rho\frac{(x-\mu_x)}{\sigma_x}\frac{(y-\mu_y)}{\sigma_y}\right) \quad (9)$$

In the present studies, μ_x = transformed population mean for unaffected or affected pregnancies for analyte PAPP-A; μ_y = transformed population mean for unaffected or affected pregnancies for analyte free ß-hCG; σ_x = standard deviation of the transformed population for unaffected or affected pregnancies for analyte PAPP-A; σ_y = standard deviation of the transformed population for unaffected or affected pregnancies for analyte free ß-hCG; x = transformed sample value, analyte PAPP-A; y = transformed sample value, analyte free ß-hCG; ρ = correlation coefficient between transformed x and y for unaffected and affected pregnancies.

When three variables are used instead of two variables above, the distribution function is set out as follows, where variable μ_1, μ_2, μ_3 represent three different maternal serum markers, in our cases, there are AFP, uE3 and free ß-hCG for the likelihood calculation in second trimester of gestation.

$$\mu = \begin{bmatrix} \mu_1 \\ \mu_2 \\ \mu_3 \end{bmatrix} \quad (10)$$

and the covariance matrix is,

$$\sum = \begin{bmatrix} \sigma_{11}^2 & \sigma_{12}^2 & \sigma_{13}^2 \\ \sigma_{21}^2 & \sigma_{22}^2 & \sigma_{23}^2 \\ \sigma_{31}^2 & \sigma_{32}^2 & \sigma_{33}^2 \end{bmatrix} \quad (11)$$

Then, the determinant of the function can be expressed by replacing the three previous establish relationships, equation 13, equation 14 and equation 15 into the covariance matrix, after simplified, as follows,

$$Det(m_2) = \sigma_{11}^2\sigma_{22}^2\sigma_{33}^2(1-\rho_{23}^2-\rho_{12}^2+2\rho_{12}\rho_{13}\rho_{23}-\rho_{13}^2) \quad (12)$$

Where the three previous establish relationships are shown as follows,

$$\sigma_{12}^2 = \rho_{12}\sigma_{11}\sigma_{22} \quad (13)$$

$$\sigma_{13}^2 = \rho_{13}\sigma_{11}\sigma_{33} \quad (14)$$

$$\sigma_{23}^2 = \rho_{23}\sigma_{22}\sigma_{33} \quad (15)$$

By replacing all the equations above and simplified the function, the inversed of $\sum$ and absolute of the covariance matrix are shown as follows,

$$\sum^{-1} = \frac{1}{\sigma_{11}^2\sigma_{22}^2\sigma_{33}^2(1-\rho_{23}^2-\rho_{12}^2+2\rho_{12}\rho_{13}\rho_{23}-\rho_{13}^2)}\begin{bmatrix} A_1 & A_4 & A_7 \\ A_2 & A_5 & A_8 \\ A_3 & A_6 & A_9 \end{bmatrix} \quad (16)$$

Where A_i denotes,

$$A_1 = \sigma_{22}^2\sigma_{33}^2(1-\rho_{23}^2) \quad (17)$$

$$A_2 = -\sigma_{33}^2\sigma_{11}\sigma_{22}(\rho_{12}-\rho_{13}\rho_{23}) \quad (18)$$

$$A_3 = \sigma_{22}^2\sigma_{11}\sigma_{33}(\rho_{12}\rho_{23}-\rho_{13}) \quad (19)$$

$$A_4 = -\sigma_{33}^2\sigma_{11}\sigma_{22}(\rho_{12}-\rho_{13}\rho_{23}) \quad (20)$$

$$A_5 = \sigma_{11}^2\sigma_{33}^2(1-\rho_{13}^2) \quad (21)$$

$$A_6 = \sigma_{11}^2\sigma_{22}\sigma_{33}(\rho_{23}-\rho_{13}\rho_{12}) \quad (22)$$

$$A_7 = \sigma_{22}^2\sigma_{11}\sigma_{33}(\rho_{12}\rho_{23}-\rho_{13}) \quad (23)$$

$$A_8 = -\sigma_{11}^2\sigma_{22}\sigma_{33}(\rho_{23}-\rho_{12}\rho_{13}) \quad (24)$$

$$A_9 = \sigma_{11}^2\sigma_{22}^2(1-\rho_{12}^2) \quad (25)$$

$$\left|\Sigma^{\frac{1}{2}}\right| = \sqrt{Det(m)} = \sigma_{11}\sigma_{22}\sigma_{33}\sqrt{(1-\rho_{23}^2-\rho_{12}^2+2\rho_{12}\rho_{13}\rho_{23}-\rho_{13}^2)} \quad (26)$$

Next, trivariate distribution function will be derived by applying all the parameters back into the general multivariate normal distribution equation 1, the explicit expression for the trivariate Gaussion is shown in equation 27, take special note that when replacing the term *(x – μ)* in equation 1, it will need to be converted into three variables before simplifying the function, as shown in equation 28 and 29.

$$f(x,y,z) = \frac{1}{(2\pi)^{3/2}\sigma_x\sigma_y\sigma_z|\Sigma|^{1/2}xyz}\exp\left\{-\frac{1}{2}(x-\mu)^1\Sigma^{-1}(x-\mu)\right\} \quad (27)$$

$$(x-\mu)^1 = (x_1-\mu_1, x_2-\mu_2, x_3-\mu_3) \quad (28)$$

$$(x-\mu) = \begin{bmatrix} x_1-\mu_1 \\ x_2-\mu_2 \\ x_3-\mu_3 \end{bmatrix} \quad (29)$$

Finally, the trivariate log normal distribution can be utilized as the formula in equation 30, to calculate the combined likelihood ratio of three serum markers including AFP, uE3 and free ß-hCG in second trimester.

$$f(x,y,z) = \frac{1}{(2\pi)^{3/2}\sigma_x\sigma_y\sigma_z\Delta^{1/2}xyz}\exp\left\{-\frac{1}{2}\sum_{j=1}^{3}\sum_{k=1}^{3}A_{jk}u_ju_k\right\} \quad (30)$$

where *f(x, y, z)* is the probability of the (MoM) values for the combination of three maternal serum markers, three different MoM values of the sample are indicated by *x, y* and *z*, μ_x = transformed population mean for unaffected or affected pregnancies for analyte AFP; μ_y = transformed population mean for unaffected or affected pregnancies for analyte uE3; μ_z = transformed population mean for unaffected or affected pregnancies for analyte free ß-hCG; σ_x = standard deviation of the transformed population for unaffected or affected pregnancies for analyte AFP; σ_y = standard deviation of the transformed population for unaffected or affected pregnancies for analyte uE3; σ_z = standard deviation of the transformed population for unaffected or affected pregnancies for analyte free ß-hCG; *x* = transformed sample value, analyte AFP; *y* = transformed sample value, analyte uE3; *z* = transformed sample value, analyte free ß-hCG; ρ = correlation coefficient between transformed *x*, y and *z* for unaffected and affected pregnancies.

The parameter of A_{jk}, u_j and u_k are defined as below,

$$u_1 = \frac{\ln x - \mu_x}{\sigma_x} \quad (31)$$

$$u_2 = \frac{\ln y - \mu_y}{\sigma_y} \quad (32)$$

$$u_3 = \frac{\ln z - \mu_z}{\sigma_z} \quad (33)$$

$$\Delta = 1-\rho_{yz}^2-\rho_{xz}^2-\rho_{xy}^2+2\rho_{yz}\rho_{xz}\rho_{xy} \quad (34)$$

$$A_{11} = (1-\rho_{yz}^2)\Delta^{-1} \quad (35)$$

$$A_{22} = (1-\rho_{xz}^2)\Delta^{-1} \quad (36)$$

$$A_{33} = (1-\rho_{xy}^2)\Delta^{-1} \quad (37)$$

$$A_{12} = A_{21} = (\rho_{xz}\rho_{yz}-\rho_{xy})\Delta^{-1} \quad (38)$$

$$A_{13} = A_{31} = (\rho_{xy}\rho_{yz}-\rho_{xz})\Delta^{-1} \quad (39)$$

$$A_{23} = A_{32} = (\rho_{xy}\rho_{xz}-\rho_{yz})\Delta^{-1} \quad (40)$$

Based on the derived equations above, we can observe that there are four parameters are not found in the bivariate case compared to the trivariate function, which are the mean value of z (μ_z), the standard deviation of z (σ_z), the correlation coefficient between *x* and *z* (ρ_{xz}) and between *y* and *z* (ρ_{yz}).

Same calculation using equation 30 is done twice in normal and abnormal pregnancies respectively at particular maternal age, in order to produce the probability of $f_{affected}(x, y, z)$ and $f_{unaffected}(x, y, z)$. The $f_{affected}(x, y, z)$ reflects to the used maternal serum markers collected from the found abnormal pregnancies, which the concentration of serum markers tested from maternal bloods are remains at abnormal level. Where else $f_{unaffected}(x, y, z)$ is the calculation based on several maternal serum markers concerned from normal pregnant women at the same maternal ages for risk calculation. The results of both $f_{affected}(x, y, z)$ and $f_{unaffected}(x, y, z)$ evaluate the probability of the MoM values for the combination of *x, y* and *z* tested belongs to the trivariate lognormal distribution in unaffected or trisomy pregnancies respectively. The ratio of the two probabilities is termed as likelihood ratio (*LR*) that reveals the risk of fetal with trisomy.

$$LR = \frac{f_{affected}(x, y, z)}{f_{unaffected}(x, y, z)} \quad (41)$$

3 Result and Analysis

In order to calculate the absolute risk of trisomy 21, two major parameters must be taken into account in the developed software, which are the background risk and the likelihood ratio. The likelihood ratio or *LR* can be obtained based on the derived bivariate and trivariate distribution algorithms in the previous section. Where else the background risk or also known as priori risk is depends on the maternal age and previous affected pregnancies and multiplied by a series of factors that rely on maternal serum markers measurements. Equation 42 shows that absolute risk can be calculated by multiplying maternal age related-risk with the previous obtained likelihood ratio.

$$Risk = A * LR \quad (42)$$

Where *Risk* = the absolute risk of fetus with trisomy 21, *A* = the maternal age related-risk, *LR* = the likelihood ratio of markers concentration. If a woman had a previous trisomy pregnancy, the recurrent risk is higher than the maternal age about 0.75 percent in the subsequent pregnancy. Therefore, equation 43 can be utilized by summing a value 0.75 with the maternal age related-risk in advanced before the multiplication of the likelihood.

$$Risk = (A + P) * LR \quad (43)$$

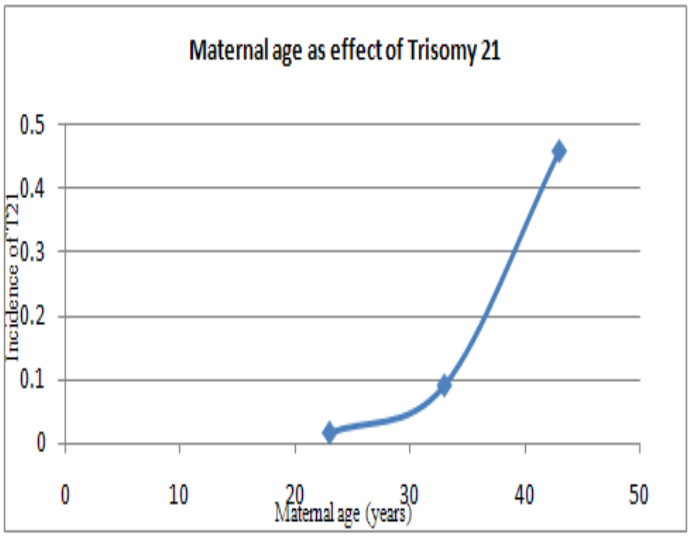
Where P = 0.75 that denotes the previous affected in percentage for the subsequent pregnancy.

In order to validate the accuracy of the present method, we have compared our finding with the published result at different particular maternal ages. The trends of the numerical distributions were found matched to the corresponding published findings. The compared parameters in the result of risk calculation are including maternal age, previous affected pregnancy and likelihood ratio of maternal serum markers during the first trimester pregnancy. Table 1 shows parts of the experimental result of the developed algorithm, where two domains maternal serum markers PAPP-A and free β-hCG are used. Table 1 can be analyzed by observing the maternal age, level of PAPP-A, free β-hCG and previous affected pregnancy separately. While increasing the maternal age or free β-hCG, and all other variables remain constant, the risk was found to increase dramatically. For example, the estimated risk was increase from 1 in 6199 at age 23 to 1 in 218 at age 43.

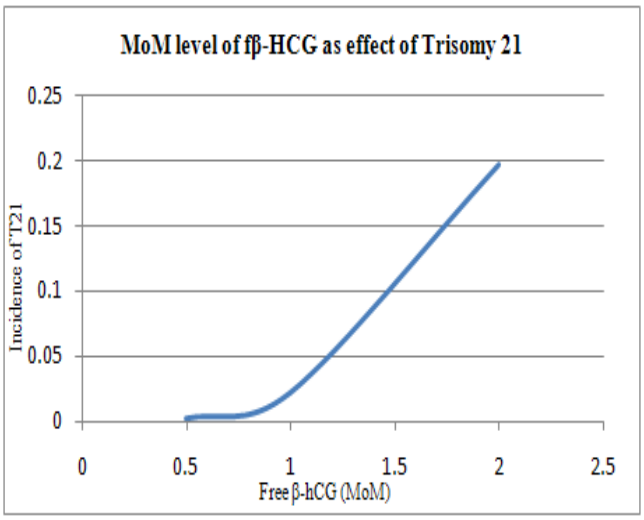
Table 1
Estimated Woman's Risk Given Her Age and Biochemical Markers Used in First Trimester

Maternal age (years)	PAPP-A (MoM)	β-hCG (MoM)	Previous affected pregnancy	Estimated Risk	Published Risk
23	1.0	1.0	0	1 in 6199	No data
33	1.0	1.0	0	1 in 2465	No data
43	1.0	1.0	0	1 in 218	No data
35	0.5	1.0	0	1 in 705	1 in 703
35	1.0	1.0	0	1 in 4497	1 in 4479
35	2.0	1.0	0	1 in 28666	1 in 28554
35	1.0	0.5	0	1 in 39982	1 in 39825
35	1.0	1.0	0	1 in 4497	1 in 4479
35	1.0	2.0	0	1 in 506	1 in 504
35	1.0	1.0	0	1 in 1652	No data
35	1.0	1.0	1	1 in 424	No data

Fig. 7 illustrated the effect of maternal age to the risk of trisomy based on the developed algorithm and Fig. 8 displays the influences of free β-hCG at different MoM level. Both of the curvatures act in a positive exponential trend.



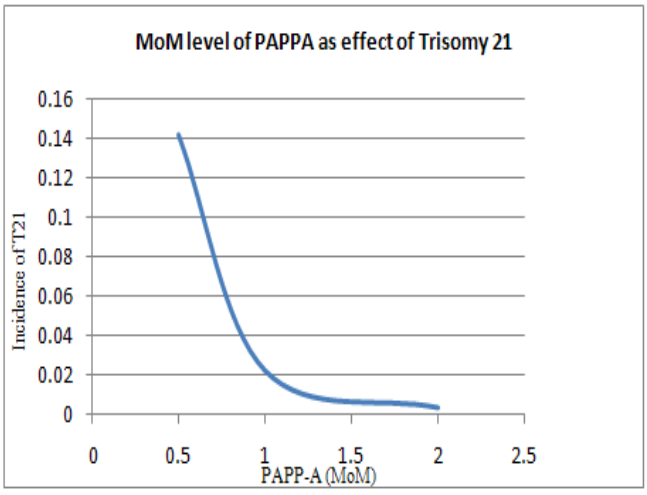
*Risk in Percentage = incidence * 100*
Fig. 7 Maternal age as effect of trisomy 21



*Risk in Percentage = incidence * 100*

Fig. 8 MoM level of free β-hCG as effect of trisomy 21

However, increasing of PAPP-A acts in the reverse way where it will decrease the risk of trisomy significantly, as shown in Fig. 9 below. It appears from our results and those from retrospective studies that combining of free β-hCG and PAPP-A with maternal age by using bivariate distribution may be as effective algorithm for chromosomal abnormalities screening as the present maternal screening programs performed during first trimester. This screening result can be further improved by ultrasound marker measurement of nuchal translucency thickness that we have done on previous project.



*Risk in Percentage = incidence * 100*

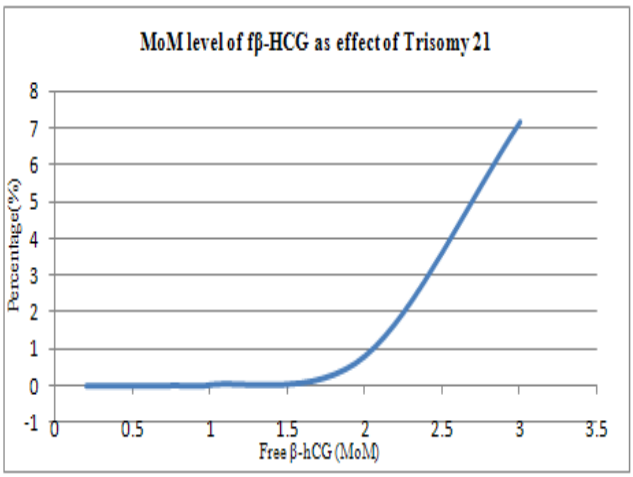
Fig. 9 MoM level of PAPP-A as effect of trisomy 21

The performance of the derived trivariate distribution was also utilizing thoroughly by analyzing the resultant trend of the produced risk. Since we proposed to use trivariate distribution, three different independent maternal serum markers including AFP, free β-hCG and uE3 are required for the second trimester pregnancy screening. Table 2 indicates the estimated risk of chromosomal abnormalities corresponding to the combination of three serum markers above. While increasing the concentration of free β-hCG in MoM and two others markers remain constant, it shows severe increment risk of chromosomal abnormalities. Nevertheless, both markers of uE3 and AFP producing a negative exponential curvature graph with the increasing of concentration in MoM.

Table 2
Estimated Woman's Risk Given Her Age and Biochemical Markers Used in Second Trimester

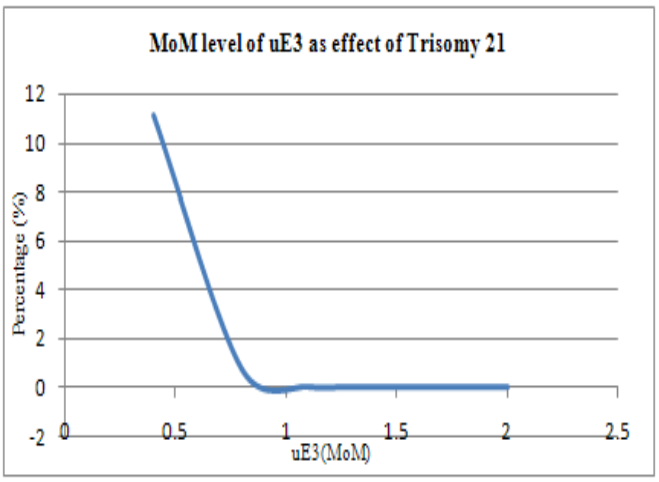
Maternal age (years)	f β-hCG (MoM)	Ue3 (MoM)	AFP (MoM)	Estimated Risk
35	0.2	1.0	1.0	1 in 1000000
35	0.8	1.0	1.0	1 in 9207
35	1.0	1.0	1.0	1 in 3442
35	2.0	1.0	1.0	1 in 123
35	3.0	1.0	1.0	1 in 14
35	1.0	0.4	1.0	1 in 9
35	1.0	0.8	1.0	1 in 804
35	1.0	1.1	1.0	1 in 6432
35	1.0	1.3	1.0	1 in 19348
35	1.0	2.0	1.0	1 in 342470
35	1.0	1.0	0.4	1 in 10
35	1.0	1.0	0.8	1 in 434
35	1.0	1.0	1.2	1 in 18313
35	1.0	1.0	2.0	1 in 194300

Fig. 10 displays the influences of free β-hCG collected during second trimester pregnancy at different MoM level.



*Risk in Percentage = incidence * 100*

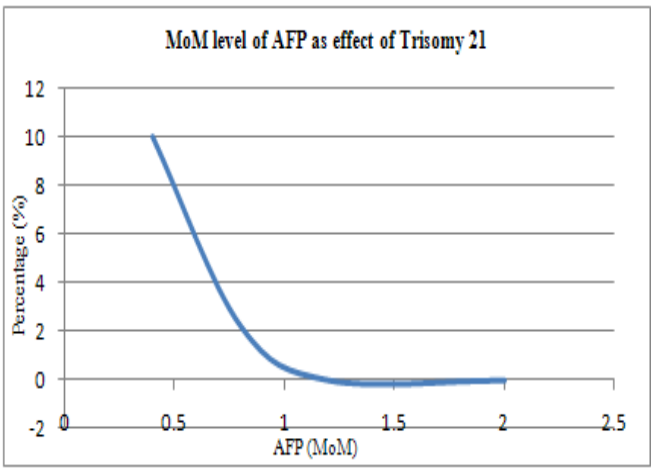
Fig. 10 MoM level of β-hCG as effect of trisomy 21



*Risk in Percentage = incidence * 100*

Fig. 11 MoM level of uE3 as effect of trisomy 21

Fig. 11 and Fig. 12 displays the influences of free uE3 and AFP collected during second trimester pregnancy at different MoM level. Our algorithm confirms the trend of serum markers behavior during early pregnancy screening. There are a number of advantages in obtaining an early diagnosis and earlier termination of the pregnancy can be performed if severe affected cases were found. Present results can also be served as the additional factors together with ultrasound screening findings for absolute risk consideration.



*Risk in Percentage = incidence * 100*

Fig. 12 MoM level of AFP as effect of trisomy 21

4 Conclusion

We have proposed a new mathematical algorithm which combines three maternal serum markers using trivariate lognormal distribution to calculate the risk of trisomy pregnancy. From this method we are able to further increase the reliability of risk assessment using three maternal serum markers, instead of using two maternal markers as usual. Moreover, future work can be concentrated on optimizing the performance by additional useful biochemical markers or maternal data. Findings showed that the system is able to provide consistent and reproducible results.

ACKNOWLEDGMENTS

The authors would like to express our thankfulness to Health Centre, Universiti Teknologi Malaysia for contributing us the maternal data, and Ministry of Science, Technology and Innovation (MOSTI), Malaysia for supporting and funding part of this study under vote 79327. Our appreciation also goes to the progressive healthcare and human development research group members for their ideas and comments on this paper.

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