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Maternal Serum Screening for Down Syndrome: Effect of Different Set of Distribution Parameters on Efficacy and Reliability of System

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Abstract: The researchers tried to use the result of multiple markers assays in maternal serum to establish a mathematical method for calculating the risk of carrying a fetus with Down syndrome. This screening method is dependent on distribution of few parameters. We investigated the effect of different set of parameters on screening system. The most popular protocol for triple marker screening of Down syndrome is based on multivariate gaussian distribution. This study has been applied in a private medical laboratory. The result of different set of distribution of parameters on efficacy and reliability of system is investigated by graphical display and simulation by monte carlo method. In some cases, different sets of parameters in protocols make calculated risk inappropriately different. In some ranges of biochemical markers, the behavior of system violates their general trends on risk calculation. We found inherent error in models. This could be explained by interaction and value of distribution of parameters. We show effect of distribution of parameters in mathematical model, estimate efficacy of system and also emphasize on quality of parameters.

Key words: Serum screening protocols, prenatal diagnosis, distribution parameters, Down syndrome, MSAFP, Iran

INTRODUCTION

Down syndrome (trisomy 21) is one of the most common causes of genetically inherited mental retardation. Besides experiencing varying degrees of mental retardation, life of many of these patients is complicated by various medical problems. Emotional and financial burden of managing these handicapped children makes prenatal diagnosis of Down syndrome desirable for families, obstetricians and community (health) authorities.

The overall probability of Down syndrome is approximately 1 in 700 live births in general population. This risk is positively correlated with maternal age (Hall, 2000). Definite diagnosis of an affected fetus with Down syndrome is possible with amniocentesis and karyotyping. Usually this option is offered to mothers >35 years age whose individual risk is sufficiently high (> = 1:270 in the second trimester) (Palomaki and Haddow, 1987). For younger mothers less invasive and cost effective screening protocols are available. As a general rule in screening policies, diagnostic procedures such as amniocentesis and karyotyping are preserved as second step for higher risk group. These screening protocols reevaluate age related risk of having affected fetus according to remarkable difference of concentration of serum biochemical markers of mothers carrying unaffected and Down syndrome babies (Wald *et al*., 1988a). The most common and well-studied markers are Á-Feto Protein (AFP), Chorionic Gonadotrophine (CG) and Unconjugated Esteriol (UE3) (Forest *et al*., 1995). Combination of biochemical markers will give better risk estimation. For example using AFP alone has detection rate of 33% which is poor. Triple markers (AFP, CG and UE3) has detection rate of 60% of affected pregnancies with the same false positive rate of 5% (Wald *et al*., 1988a). The aim of this study was to review, some protocols of screening methods and effect of some recommended distribution parameters on the efficacy of system.

MATERIALS AND METHODS

Screening for Down's syndrome and NTD (Neural Tube Defect) has been applied in Chizar laboratory (Tehran, Iran) since 1997. Between 14-22 weeks of pregnancy a blood sample was obtained from each case. At the time of blood sampling if requesting physician had not provided sufficient clinical information, patients were asked to fill a questionnaire for necessary information for risk calculation. AFP, CG and UE3 concentration were measured in serum. A computerized algorithm in Excel software (Office 97, Microsoft Corp, USA) was developed

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Table 1: Distribution parameters for down syndrome and unaffected pregnancies

	Unaffected				Down			
	Weight adjusted		Not weight adjusted		Weight adjusted		Not weight adjusted	
Parameters	Date	Scan	Date	Scan	Date	Scan	Date	Scan
Standard deviation								
AFP	0.1936	0.1789	0.1986	0.1902	0.1965	0.1821	0.2015	0.1932
UE3	0.1374	0.1102	0.1391	0.1138	0.1462	0.1210	0.1478	0.1243
hCG	0.2336	0.2239	0.2401	0.2330	0.2606	0.2520	0.2665	0.2601
Correlation coefficient								
AFP, UE3	0.2451	0.090	10.2853	0.1291	0.3008	0.1770	0.3359	0.2048
AFP, hCG	-0.0199	0.0596	0.0327	0.1119	0.1282	0.2148	0.1681	0.2472
UE3, hCG	-0.1688	-0.0586	-0.1423	-0.0244	-0.3860	-0.0470	-0.3565	-0.3073
Mean								
AFP	0	-	-	-	-0.1427	-	-	-
UE3	0	-	-	-	-0.1411	-	-	-
hCG	0				0.3023	-	-	-

Distribution Parameters from Wald *et al.* (1994)

that had the ability to incorporate the results of Maternal Serum AFP (MSAFP), Maternal Serum CG (MSCG) and Maternal Serum UE3 (MSUE3) assays with maternal age (after correction for weight) and the state of multiple pregnancy and diabetes mellitus.

Mathematical basis of MSAFP screening: The concentrations of biochemical markers in the maternal serum screening depends on gestational week, population based studies and reference value estimated by laboratory. Using the ratio obtained from patient results divided by normal median estimated by laboratory for each corresponding week will normalize this effect and allows calculation of risk by applying only one algorithm.

MoMs (Multiple of Median) of markers enter into probability model for risk estimation (Neveux *et al*., 1996). The probability model of MoMs after logarithmic transformation fits Normal (Gaussian) distribution in specified ranges: 0.3-3.3 MoM for AFP, 0.5-2 MoM for UE3 and 0.2-5 MoM for CG (Wald *et al*., 1994).

Multivariate Gaussian distribution model could be used for estimation of probability of combination of multiple markers (Wald *et al*., 1988a). The introduction of >2 variables into Gaussian distribution results in conceptual difficulty which could be overcome by mathematical analysis using matrix algebra (Reynolds and Penny, 1989). The population distribution parameters in the terms of mean and standard deviation, correlation coefficient defined in a 3×3 matrix.

The result of markers in MoM interacts with parameter matrix by matrix multiplication of (1,3)×(3,3)×(3,1) which results 1×1 matrix which is a number. Multivariate Gaussian distribution for triple markers handles this calculation in a four-dimension space which the axes are AFPMoM, CGMoM, UE3MoM and f (frequency). For each patient model solves twice, one time according to unaffected and then for Down parameters. The Down's syndrome distribution parameters in this mathematical model show how many affected pregnancies (fD) could have pattern of triple markers. The distribution parameters also calculate the same parameters for unaffected pregnancies (fU) and estimate how many unaffected pregnancies show this pattern of markers. The ratio obtained from these two figures is called Risk Modifiers (RM) for condition. Multiplication of age risk in RM will result final risk (odd) of Down's syndrome for patient. For execution of screening model the popular distribution parameters which were provided by Wald *et al*. (1988a) were used (Ashwood, 1999). This study provided 4 sets of parameters (Table 1).

In specified range of MoMs, protocols plotted by Mathematica software (Wolfram Research Inc. USA). Because of difficulty in visual display of a four-dimensional system as whole, the system was plotted in consecutive graphs in software. Performance of each model was examined by Monte Carlo simulation. Randomized numbers were generated by Mathematica software from hypothetical normal distribution of each markers for Down's and unaffected populations individually. These randomly simulated cases used to estimate the test specification of protocol in terms of false positive and sensitivity.

RESULTS

RM and final risk for Down's syndrome calculated by each set of parameters were different. In some cases, these differences are so great in degree that it jumped around critical point (1.270) and compromised decision making. For example considering two set of parameters, weight adjusted date and weight adjusted Scan, AFPMoM = 0.80, CGMoM = 0.40, UE3MoM = 0.55, the calculated RM based on weight adjusted scan equaled

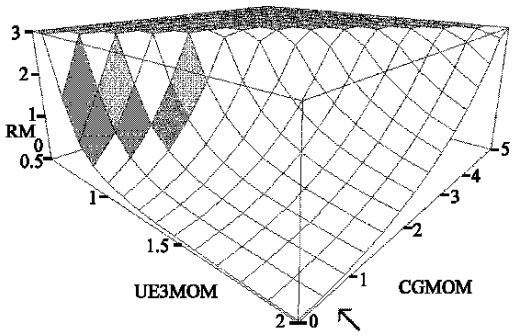

Fig. 1: Effect of distribution parameters on maternal serum screening. The graphical display of weight adjusted Scan model in AFPMoM (A-Feto-Protein Multiple of Median) of 0.3. Arrow shows that RM Risk Modifier) declines with decrease in CGMoM (Chorionic Gonadotropin Multiple of Median) and increase of UE3MoM (Unconjugated Estriol Multiple of Median)

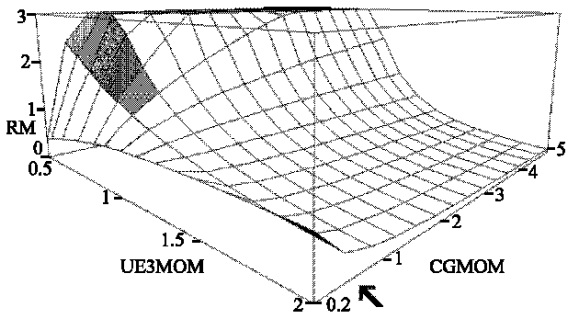

Fig. 2: Effect of distribution parameters on maternal serum screening. The graphical display of weight adjusted data model in AFPMoM (A-Feto-Protein Multiple of Median) of 0.3. Arrow shows that RM Risk Modifier) declines with decrease in CGMoM (Chorionic Gonadotropin Multiple of Median) and increase of UE3MoM (Unconjugated Estriol Multiple of Median)

0.5252 but based on weight adjusted date it equaled 0.05697 consider that for a patient with age risk of 1.75, the scan risk should be 1.143 and date risk should be 1.1316. Also, we noted that around these range of markers trend of concentration of markers on risk is become reveres for weight unadjusted scan and date model both weight adjusted and unadjusted. Fixing two markers AFP and CG and increasing UE3 increases risk. For example in the earlier mentioned case rising UE3MoM-0.60 results RM for weight adjusted date of 0.06357. The visual display of system is provided as a graph of RM for Down's syndrome (fD/fU). Fixing a value for AFPMoM then their axis are CGMoM, UE3MoM and RM. Figure 1 shows the graph of date system in AFPMoM = 0.3. For comparison, Fig. 2 is provided from Scan system for the same AFPMoM.

DISCUSSION

The most common cause of positive screen result is wrong estimation of gestational week. LMP date is a default estimator but if 10-14 days of discrepancy exists then instead of LMP an estimation of gestational age by Ultra Sonography (Scan) must be used. Ultra Sonography is a better estimator for gestational week and is recommended for a definite judgment. This fact is highlighted specifically by Wald *et al.* (1994) and also in the simulation, Scan model shows a better performance compared with date. With maternal age and all the other three markers when gestational age is estimated by Ultra Sonography the detection rate is increased from 58-67% while holding the false positive at 5%, respectively (Wald *et al.*, 1992).

If the gestational age estimated by scan and LMP are the same, we would expect to have more reliable results, although somehow different results are achieved from both protocols. As the scan protocol seems to be a more accurate one, it seems appropriate to choose it. But on the other hand this suggestion is complicated by great discrepancy between obtained risks by two models. In some cases calculated, risk means positive and in another it is negative.

There is no doubt that each model must be able to produce reliable and trustworthy results based on the population reference value. Based on population studies and statistical concepts one set of markers can be interpreted differently in different populations. What made us suspicious about error in the background of these calculations was not mere existence of discrepancy but actually violation of general trend of markers on estimating the final risk.

It is generally, accepted that trends of concentration of each markers on risk are based on universal rules. In this regard if increase in AFP or UE3 and decrease in CG adds on risk of Down's syndrome (RM) this is against principles of protocol and original observation of pattern of concentration of AFP (Merkatz *et al.*, 1984), CG (Bogart *et al.*, 1987) and UE3 (Canick *et al.*, 1988; Wald *et al.*, 1988b).

The example in result shows that rise of UE3MoM increases RM. It apparently violates general trend of marker in risk estimation. This abnormal trend of systems could be visualized in graphical display of mathematical software. Scan and date is a graphical output of software

Mathematica from (weight adjusted scan and weight adjusted date). Date is well located in one of these areas which we could blame system for violation of trends. In these graphs, we chose AFPMoM = 0.3. Graph date shows that increase of UE3MoM axis causes increase in RM also in the axis of CGMoM slight increase in RM for lowering values of CGMoM (arrow).

This graph reveals that odd behavior of system is not exceptional and is a continuous bias in system. In comparison. Scan is in the same area of system and shows smooth systematic increase in Down probability (RM) with increase of CG and decrease of UE3, the healthy expected behavior of system.

Putting this visual display with lower performance of those set of parameters points to us the effect of parameters. Some manipulation on parameters could correct this effect on system and improves theoretical performance. As an example only changing correlation of coefficient of UE3 and CG for Down's pregnancy population in weight adjusted date (of course toward zero) eliminates this bias of system and clears abnormal areas in graphs. Furthermore with this experimental, maneuver on this parameter the performance of system improves. Monte Carlo simulation before and after this manipulation shows approximately 5% rise in sensitivity of weight adjusted date model. There are many discussions concerning the effect of the means and standard deviations but little interest has been shown in the effect of the correlation of coefficients between markers in this system (Dunstan *et al*., 1999).

The experimental manipulation on correlation of coefficient is a good example for revealing the effect and importance of parameters on efficacy of system. Researchers addressed increase in detection rate of system with changing parameters and proposed methods to extract better parameters (Cuckle, 1995; Williams *et al*., 2000). At the beginning of set up of the system it is not possible for each respective laboratory to perform population based studies so, they generally use one of the published parameter sets for risk estimation (Cuckle, 1995).

We have the same concern about quality of parameters as Williams *et al*. (2000). Researchers recommend that each laboratory calculates its own distribution parameters from local population and assay methods (Forest *et al*., 1995). In this regard, it adds on the importance of the role of the verification of the operating parameters of mathematical and statistical tools in screening system. Laboratory has the pivotal role and responsibility in the maternal serum screening. Statistical analysis and modeling are laboratory diagnostic tools and could also be a source of significant error so we need some kind of verification and quality control. We did not attempt to show how we might overcome the sub-optimization inherent in the screening process.

CONCLUSION

In present study, tries to move one step forward toward realization of existing quality problems in parameters and importance of all distributaries parameters. This analysis influences on any possible precautions about these parameters and emphasizes that however, the parameters are originated we have to verify them systematically.

REFERENCES

Ashwood, E.R., 1999. Clinical Chemistry of Pregnancy. In: Textbook of Clinical Chemistry, Burtis, C.A. and E.R. Tietz, WB Saunders, Philadelphia, pp: 1736-1775.

Bogart, M.H., M.R. Pandian and O.W. Jones, 1987. Abnormal maternal serum chorionic gonadotropin levels in pregnancies with fetal chromosome abnormalities. Prenat. Diagn., 7: 623-630.

Canick, J.A., G.J. Knight, G.E. Palomaki, J.E. Haddow, H.S. Cuckle and N.J. Wald, 1988. Low second trimester maternal serum unconjugated oesteriol in pregnancies with Downs syndrome. BJOG Int. J. Obstet. Gynaecol., 95: 330-333.

Cuckle, H., 1995. Improved parameters for risk estimation in Downs syndrome screening. Prenat. Diagn., 15: 1057-1065.

Dunstan, F.D., T.C. Iles, A.B. Nix and K. Williams, 1999. Correlation of marker values in Downs syndrome screening: The effect of dating error. Ann. Clin. Biochem., 36: 460-467.

Forest, J.C., J. Masse, F. Rousseau, J.M. Moutquin, N.A. Brideau and M. Belanger, 1995. Screening for Down syndrome during the first and second trimesters: Impact of risk estimation parameters. Clin. Biochem., 28: 443-449.

Hall, J.G., 2000. Chromosomal Clinical Abnormalities. In: Nelson Textbook of Pediatrics, Behrman, R.E. and R.M. Kliegman and H.B. Jenson (Eds.). WB Saunders, Philadelphia, pp: 325-332.

Merkatz, I.R., H.M. Nitowsky, J.N. Macri and W.E. Johnson, 1984. An associatin between low maternal serum a-fetoprotein and fetal chromosomal abnormalities. Am. J. Obstet. Gynecol., 148: 886-894.

Neveux, L.M., G.E. Palomaki, D.A. Larrivee, G.J. Knight and J.E. Haddow, 1996. Refinements in managing maternal weight adjustment for interpreting prenatal screening results. Prenat. Diagn., 16: 1115-1119.

Palomaki, G.E. and J.E. Haddow, 1987. Maternal serum alpha-fetoprotein, age and Down syndrome risk. Am. J. Obstet. Gynecol., 156: 460-463.

Reynolds, T.M. and M.D. Penny, 1989. The mathematical basis of multivariate risk screening: With special reference to screening for Downs syndrome associated pregnancy. Ann. Clin. Biochem., 27: 452-458.

Wald, N.J., H.S. Cuckle, J.W. Densem, A. Kennard and D. Smith, 1992. Maternal serum screening for Downs syndrome: The effect of routine ultrasound scan determination of gestational age and adjustment for maternal weight. Br. J. Obstet. Gynaecol., 99: 144-149.

Wald, N.J., H.S. Cuckle, J.W. Densem, K. Nanchahal and J.A. Canick *et al*., 1988a. Maternal serum unconjugated oesteriol as an antenatal screening test for Downs syndrome. Br. J. Obstet. Gynaecol., 95: 334-341.

Wald, N.J., H.S. Cuckle, J.W. Densem, K. Nanchahal and P. Royston *et al*., 1988b. Maternal serum screening for Downs syndrome in early pregnancy. BMJ, 297: 883-887.

Wald, N.J., J.W. Densem, D. Smith and G.G. Klee, 1994. Four marker serum screening for Downs syndrome. Prenat. Diagn., 14: 707-716.

Williams, K.L., B.A.J. Nix and F.D.J. Dunstan, 2000. Effect of screening algorithm, parameter values and median smoothing on patient-specific risk estimates for Downs syndrome screening. Ann. Clin. Biochem., 37: 165-173.