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CLINICAL ARTICLE

The effects of analytical factors on second trimester risk estimations

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Abstract

Objective: Triple test with measured maternal serum α-fetoprotein, human chorionic gonadotropin, and unconjugated estriol combination as a routine procedure for fetal Down's syndrome, trisomy 18 and neural tube defect screening has some intrinsic problems, such as precision. The aim of this study was to evaluate the effect of analytical variation of triple test on prenatal risk estimation. *Method:* Five different serum pools were prepared and triple test was performed seven times for within run and five times for between run precision determination. *Result:* Within run and between run, precision values of risk estimations by measuring the same sample for Triple test were calculated to be 7.9—21.4% and 14.1—31.0% for trisomy 21, 13.2—23.7% and 14.2—15.1% for trisomy 18, 47.2 and 42.0 % for neural tube defect, respectively. *Conclusion:* These results demonstrated that analytical variations have great impact on second trimester risk estimation procedures; therefore, triple test analyses should be carried out in laboratories using strict internal and external quality control programs. Moreover, triple test results should always be interpreted by considering analytical and biological variations.

1. Introduction

Second trimester screening for Down's syndrome, trisomy 18 and neural tube defect (NTD) has become an important component of routine pregnancy follow up during the last few decades. Second trimester screening covers measurement

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of maternal serum α-fetoprotein (AFP), human chorionic gonadotropin (hCG), and unconjugated estriol (uE3) levels [1,2].

Second trimester risk estimation is carried out in a number of laboratories using various analytical methods and software packages; thus, both biological and analytical variations among the results of laboratories are not uncommon. Analytical imprecision in determination of the components of the triple test is generally considered to be the major cause of the higher variations observed during in risk estimation. The consequences of these variations can vary from retesting and patient anxiety to invasive procedures like amniocentesis. The variations of the test results may arise from methodological differences of immunoassays, biological changes during pregnancy, the use of different risk algorithms and quality control procedures [3]. Since analytical variations directly affect the precision of an individual risk estimation [4,5], an inadequacy in the performance of any component of the triple test may lead to invasive, expensive and even inappropriate diagnostic procedures those carrying the risk of *misconception* [6]. Accordingly, these estimations are advised to be carried out under strict quality control programs [1,2].

The contribution of methodological imprecision on risk estimations was emphasized by various authors and an increase in the analytical imprecision of any test parameter was demonstrated to lead to increased variations in the likelihood ratio of prediction rates of Down's syndrome [7—9].

Analytical variations in maternal serum concentrations and Multiple of Medians (MoM) values of AFP, hCG and uE3 from five different serum pool samples were determined for predicting their probable contribution on the second trimester risk estimations at each time point. These data were used to calculate the within run and between run coefficient of variation (%CV) rates.

2. Materials and methods

Five serum pools with different MoM values due to varying AFP, hCG and uE3 levels were prepared for simulation of medical conditions of concern:

Level-1 (for trisomy 18): low AFP, low uE3, low hCG;
Level-2 (for NTD): high AFP, low uE3, normal hCG;
Level-3 (normal level): normal AFP, high uE3, normal hCG;
Level-4 (for Down's syndrome): low AFP, low uE3, high hCG and
Level-5 (high MoM levels): high AFP, high uE3, high hCG.

All the samples were analyzed seven times in a day (within run [intraassay]) and then just once every day during five consecutive days (between run [interassay]) to determine the precision rates. The risk ratios for Down's syndrome, trisomy 18 and NTD as well as Double test (without uE3) and ULM index (hCG MoM^2 / [AFP $MoM^2 \times$ uE3 MoM])) were calculated by using Prenatal Screening Calculation Program (PRISCA, Typolog Software GmbH, Hamburg, Germany). Serum AFP and hCG were measured with an immunochemiluminescence method by ADVIA CENTAUR analyzer (Bayer Corporation Diagnostic Division, Tarrytown, NY, USA); and serum uE3 by using an active ultrasensitive unconjugated estriol radioimmunoassay kit (Diagnostic Systems Laboratories Inc, TX, USA).

This study was carried out in a large clinical laboratory following internal and external quality control programs and the results were obtained in a blind manner. The risk estimations were calculated with the assumption that serum samples were belonging to a 25-year-old woman at 17 weeks of pregnancy.

The concentrations and MoM values were shown as mean $\pm$ S.D. and %CV ((S.D. / mean) $\times$ 100) for each test. Calculated risk estimation ratios were given as mean, minimum, maximum level and CV [10].

3. Results

Serum concentrations and MoM values of AFP, hCG and uE3 as well as results of risk calculations for each sample are shown in Table 1.

Both within run and between run analyses yielded unacceptably high variations in risk estimation values for Down's syndrome, trisomy 18 and NTD. Within run and between run precision values were determined as 21.4% and 31.0% for Level-4 (for Down's syndrome), 14.2—15.1% and 23.7—13.2% for trisomy 18 (for Levels 1—4), respectively. Additionally, precision values for NTD were also found to be at high range (Table 1), and precision values for ULM index and Double test were more than 15%.

In this study, different scenarios represented in Table 2 were used to evaluate the effects of analytical variations on risk estimations. Accordingly, higher hCG and higher uE3 and lower AFP (or vice versa) determinations than actual amounts of the analytes were calculated to yield imprecise risk

Table 1 Analytical variation of all parameters

		uE3 (ng/ml) mean ± S.D. (%CV) MoM ± S.D.	AFP (ng/ml) mean ± S.D. (%CV) MoM ± S.D.	HCG (kU/L) mean ± S.D. (%CV) MoM ± S.D.	Down syndrome, mean risk ratio (min–max) (%CV)	Trisomy 18, mean risk ratio (min–max) (%CV)	NTD, mean risk ratio (min–max) (%CV)	DT, mean risk ratio (min–max) (%CV)	ULM index, mean ± S.D. (min–max) (%CV)
Level 1	Within run	0.77 ± 0.08 (10.3) 0.32 ± 0.03	27.8 ± 1.4 (5.1) 0.63 ± 0.03	15.99 ± 0.77 (4.9) 0.67 ± 0.03	1:1302 (1:1016–1:1757) (20.0)	1:389 (1:326–1:498) (14.2)		1:3765 (1:3045–1:4617) (16.6)	4.01 ± 0.96 (2.7–5.4) (24.0)
	Between run	0.76 ± 0.1 (13.6) 0.32 ± 0.04	27.8 ± 2.2 (8.0) 0.64 ± 0.05	16.29 ± 1.27 (7.8) (0.68 ± 0.05)	1:1256 (1:982–1:1925) (14.1)	1:406 (1:300–1:529) (23.7)		1:3654 (1:2561–1:5180) (28.8)	4.16 ± 1.1 (2.5–5.3) (26.58)
Level 2	Within run	1.58 ± 0.14 (8.9) 0.66 ± 0.06	109.1 ± 5.8 (5.3) 2.49 ± 0.13	31.81 ± 1.79 (5.6) 1.33 ± 0.08	1:5444 (1:4800–1:5893) (7.9)		1:94 (>1:50–1:186) (47.2)		0.47 ± 0.08 (0.4–0.6) (16.03)
	Between run	1.56 ± 0.23 (14.6) 0.65 ± 0.09	107.2 ± 6.61 (6.2) 2.45 ± 0.15	32.5 ± 2.34 (7.2) 1.36 ± 0.1	1:5072 (1:4429–1:6616) (14.1)		1:121 (1:72–1:221) (42.0)		0.54 ± 0.15 (0.3–0.7) (28.8)
Level 3	Within run	2.28 ± 0.22 (9.5) 0.95 ± 0.09	43.17 ± 2.65 (6.1) 0.99 ± 0.09	22.29 ± 1.50 (6.7) 0.93 ± 0.06	1:3201 (1:2310–1:3962) (19.7)			1:4869 (1:3391–1:6464) (21.9)	1.02 ± 0.3 (0.7–1.6) (29.01)
	Between run	2.27 ± 0.22 (9.6) 0.94 ± 0.09	43.48 ± 4.57 (10.5) 0.99 ± 0.1	22.55 ± 2.11 (9.4) 0.95 ± 0.09	1:3147 (1:2307–1:4163) (23.2)			1:4759 (1:3247–1:6599) (27.9)	1.06 ± 0.36 (0.7–1.6) (33.7)
Level 4	Within run	0.75 ± 0.09 (11.7) 0.31 ± 0.04	21.96 ± 1.83 (8.3) 0.50 ± 0.04	50.13 ± 3.48 (6.9) 2.1 ± 0.15	1:62 (>1:50–1:102) (21.4)	1:3613 (1:2974–1:4483) (15.1)		1:252 (1:215–1:367) (13.9)	63.8 ± 14.9 (41–89) (23.2)
	Between run	0.73 ± 0.1 (13.8) 0.30 ± 0.04	22.52 ± 2.3 (10.2) 0.51 ± 0.05	50.03 ± 3.23 (6.4) 2.1 ± 0.14	1:63 (>1:50–1:120) (31.0)	1:3703 (1:3026–1:4221) (13.2)		1:258 (1:215–1:367) (24.3)	68.3 ± 20.5 (24.1–92.3) (30.1)
Level 5	Within run	3.76 ± 0.4 (10.5) 1.57 ± 0.17	54.87 ± 4.0 (7.3) 1.25 ± 0.09	35.01 ± 2.19 (6.2) 1.47 ± 0.09	1:3182 (1:2797–1:3920) (13.1)			1:2791 (1:2223–1:3970) (21.6)	0.94 ± 0.18 (0.7–1.1) (19.2)
	Between run	3.84 ± 0.35 (9.2) 1.6 ± 0.15	55.18 ± 0.35 (9.1) 1.26 ± 0.11	32.48 ± 2.59 (7.8) 1.36 ± 0.11	1:3608 (1:3225–1:4303) (12.5)			1:3406 (1:2921–1:4581) (18.3)	0.76 ± 0.15 (0.6–0.9) (19.9)

NTD: neural tube defect, DT: double test, AFP: α-fetoprotein, hCG: human chorionic gonadotrophine, uE3: unconjugated estriol.

Table 2 The effect of analytical errors in different scenarios

	MoM values			Error levels (%)	Risk ratio for Trisomy 21	Alteration in risk ratio (%)
	AFP	hCG	uE3			
1.	1	1	1	0	1:3390	–
2.	0.95	0.95	0.95	5	1:3296	2.9
3.	1.05	0.95	1.05	5	1:4244	−20.1
4.	0.95	1.05	1.05	5	1:3032	11.8
5.	1.05	1.05	1.05	5	1:3480	−12.9
6.	0.9	0.9	0.9	10	1:3315	2.3
7.	1.1	0.9	1.1	10	1:4592	−26.2
8.	0.9	1.1	1.1	10	1:2694	25.8
9.	1.1	1.1	1.1	10	1:3567	−5.0

Analytical errors for each test were selected as 5–10%.

estimations, particularly in 3rd, 4th, 7th and 8th scenarios.

4. Discussion

Recently, the analytical and administrative parts of maternal serum screening programs are satisfactorily optimized with the improvement of the performance of screening tests. This has been made feasible with the use of modern automated analytical techniques and equipment in laboratories with high throughput screening and by employing standardized quality control procedures.

Although MacRae et al. had previously reported that the contributions of likely analytical variations in hCG, AFP and uE3 determinations on the estimation of trisomy 21 are negligible [11] as well as many other articles emphasizing various conclusions on this subject, a consensus that the borderline cases are the most problematic is almost accepted by many authors [4,5,7,8].

Williams et al. concluded that the precision of borderline samples with UKNEQAS program might be found between 1:159 and 1:365 [4]. Similarly, in this study, the precision values for borderline samples with trisomy 21, trisomy 18 and NTD were found as 1:50–1:120, 1:300–1:529 and 1:72–1:221, respectively (Table 1).

The influence of within run precision values of 5 different patients on likelihood ratios of Down's syndrome was evaluated by Holding, and the variability of likelihood ratios were found as 24.6%–47.8%, despite %CV values being <10% for each test [7]. Recently, in another and large scaled study of Benn and Collins, methodological precision values of AFP alone, the double test with AFP–hCG, and the triple test with AFP–hCG–uE3 were determined and their influences on likelihood ratios were calculated; in this regard, the effect of triple test CV variations on likelihood ratio was found to be ranging between 13.56% and 23.06% [8].

It is of particular significance in this regard that the analytical variations represented Table 2 affect the risk ratios differently in diverse combinations (especially in case of positive error in hCG and uE3, and negative error in AFP). Furthermore, systematical errors should be taken into consideration in this regard when evaluating the internal quality control procedures and, one should keep in mind that analytical imprecision value of an individual analyte may not lead to a predicted imprecision of risk ratio in every case (Table 2 and Fig. 1).

One of the main goals of this study was to increase the awareness of both the physicians and laboratory staff about relatively higher precision values (>20%) of triple test and its probable consequences.

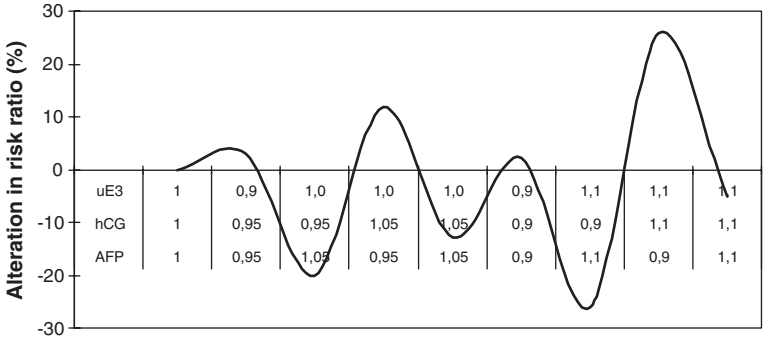

Figure 1 The effects of analytical errors in different scenarios or patients.

In this regard, the following measures are recommended for decreasing the variations in second trimester maternal serum screening test evaluations:

1. The performance of the laboratory including staff education, quality and maintenance of analytical equipment as well as chemicals, and quality management should be maintained at its best level. Particularly internal Quality Control (QC) procedures consisting of a QC material with analyte range around triple test limits should unavoidantly be used along with QC rules.
2. QC procedures with early recognizing and correcting power for errors should be preferred for triple test components.
3. Random error rate of each laboratory should have been established. If it is not possible to reduce the random error rate, re-determination of the samples with extreme values is recommended; however, retesting was expressed to be an ineffective solution of this problem by some authors [11,12].
4. Performance evaluation of a laboratory including the results as well as risk estimation procedures should regularly be monitored with a suitable external QC program.
5. Using specific formulae like Ulm Index for predicting whether the risk is age dependent or not may be helpful.
6. Finally, the limits of responsibilities regarding the analysis and interpretation of the tests as well as acquaintance and accurate information transfer to patients should be established for the staff of any medical care unit for assuring the best follow up program.

In conclusion, one must keep in mind that the second trimester risk estimation procedures are prone to analytical and biological variations and, this is of particular significance for borderline and advanced age (>35 age) pregnancies [13].

Although these probability tests are believed to be replaced by more reproducible and accurate tests in the future, at present circumstances, high rates of analytical precision in second trimester screening during risk estimation procedures of trisomy 21, 18 and NTD should be considered.

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