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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