Pak. J. Biotechnol. Vol. 7 (1-2) 91-99 (2010) ISSN. 1812-1837

EARLY DETECTION OF THE 21st CHOMOSOME TRISOMY BY AFP, β-HCG AND uE3

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ABSTRACT

Trisomy 21 is the most common autosomal chromosome anomaly. Its incidence ranges from 0.3 to 3.4 per 1000 births in different parts of the world. In Egypt, the incidence of Down syndrome has been reported to be one per 1000 births. Screening for Down syndrome through different biochemical markers is still and will be a better basic tool for detection of Down syndrome instead of invasive procedures. The aim of the presented study was to evaluate the triple screening test (using AFP, β-HCG and uE3) in prenatal diagnosis of Down syndrome through comparing the results of the test with the outcome of pregnancy. We compared these results with the results of the double test (using AFP and β-HCG) and MSAFP test to evaluate the value of combining β-HCG, alpha-fetoprotein (AFP) and unconjugated estriol with maternal age in this three-analyte maternal serum screening program for Down syndrome. Triple screening test using AFP, HCG and uE3 proved to have the upper hand over MSAFP and double test in detection of Down syndrome as it has the highest detection rate and lowest false positive rate.

INTRODUCTION

The formal story began in 1866, when a physician named John Langdon Down published an essay in England in which he described a set of children with common features who were distinct from other children with mental retardation. Down was superintendent of an asylum for children with mental retardation in Surrey, England when he made the first distinction between children who were cretins (later to be found to have hypothyroidism) and what he referred to as "Mongoloids".

Down based this unfortunate name on his notion that these children looked like people from Mongolia, who were thought then to have an arrested development. This ethnic insult came under fire in the early 1960s from Asian genetic researchers, and the term was dropped from scientific use. Instead, the condition became called "Down's syndrome." In the 1970s, an American revision of scientific terms changed it simply to "Down syndrome" while it still is called "Down's" in the UK and some places in Europe

Down syndrome is the most common autosomal chromosome anomaly. Its incidence ranges from 0.3 to 3.4 per 1000 births in different parts of the world. (1). In Egypt, the incidence of Down syndrome has been reported to be one per 1000 births (2). Physical features of Down syndrome are numerous and nearly all body organs are affected as:

1) **CNS:** Moderate-to-severe mental retardation occurs, with an intelligence quotient (IQ) of 20-85 (mean,approximately 50) (3). Hypotonia improves with age. Articulatory problems are present. Sleep apnea occurs when inspiratory airflow from the upper airway to the lungs is impeded for 10 seconds or longer (4).

2) **CVS:** Congenital heart defects are common (40-50%); they are frequently observed in patients with Down syndrome who are hospitalized, 62%, and they are a common cause of death in this aneuploidy in the first 2 years of life (5). The most common congenital heart defects are endocardial cushion defect, 43%, ventricular septal defect, 32%, secundum atrial septal defect, 10%, tetralogy of Fallot, 6%, and isolated patent ductus arteriosus, 4%, (6). About 30% of patients have several cardiac defects (6). The most common lesions are patent ductus arteriosus, 16%, and pulmonic stenosis, 9%. About 70% of all endocardial cushion defects are associated with Down syndrome (7).

3) **Hematologic System**: The relative risk of acute leukemia in the first five years of life is 56 times that of non-DS individuals. Approximately one in 150 patients develops leukemia. Acute myeloid leukemia (AML) is as common as acute lymphoid leukemia (ALL) (8). Neonatal leukemoid reactions (i.e., pseudoleukemia) are common and distinguishing this from true leukemia frequently poses a diagnostic challenge. The patient's risk of carrying hepatitis B increases if s/he was previously institutionalized (8).

4) **Others:** Hypothyroidism (16-20% of young patients), diabetes and decreased fertility occur,infectious diseases, especially pneumonia, because of impaired cellular immunity (9). Short and broad hands, clinodactyly of the fifth fingers with a single flexion crease, 20%, hyper-extensible finger joints, increased space between the great toe and the second toe, and acquired hip dislocation, 6%, are typical presentations (10). Up-slanting palpebral fissures, bilateral epicanthal folds, Brushfield spots (speckled iris), refractive errors 50% strabismus, 44%, nystagmus, 20%,blepharitis,33%, conjunctivitis, tearing from stenotic nasolacrimal ducts, congenital cataracts, 3%, pseudopapilledema, spasm nutans, acquired lens opacity, 30-60%, and keratoconus in adults are observed. Atlantoaxial instability, 14%, can result from laxity of transverse ligaments that ordinarily hold the odontoid process close to the anterior arch of the atlas (6).

The aim of this study was to evaluate the triple screening test in prenatal diagnosis of Down syndrome through comparing the results of the test with the double test (using AFP and β-HCG) or Maternal Serum Alpha-Fetoprotein Screening (MS AFP) test and evaluation of combining β-subunit of Human chorionic gonadotropin (β-HCG), alpha-fetoprotein (AFP) and unconjugated estriol with maternal age in a three-analyte maternal serum-screening program for Down syndrome.

PATIENTS AND METHODS

This study included 50 pregnant women 35 years old or older ranging from 14th to 19th week of pregnancy calculated from the 1st day of last menstrual period. They were selected from the outpatients of Sayed Galal Hospital-Azhar University, obstetrics clinic during the period from March until July 2006. All selected women were multipara, non-smokers, non-diabetic, with singleton pregnancy and normally conceived pregnancy not IVF. The selected women were subjected to laboratory assays including:

1. Measurement of maternal serum AFP, β-HCG and uncojugated estriol uE3) using Biosource ELISA kits.
2. Calculation of age risk for Down syndrome plus risk ratio using MSAFP, double test (MSAFP and β-HCG) and triple test (MSAFP, β-HCG and uE3). A cut-off value 1:270 or less was considered

screen positive (high risk). The "cut-off" median MoM values in pregnancies with Down syndrome were 0.73 (AFP), 2.02 (β-HCG) and 0.74 (uE3).

3. After birth, the outcome of pregnancy of the selected cases is evaluated clinically for Down syndrome diagnosis. The cases proven clinically to be of Down syndrome were karyotyped for confirmation of results.

RESULTS

The detection and false positive rates for triple double and MSAFP tests are shown in Figure 1 and 2.

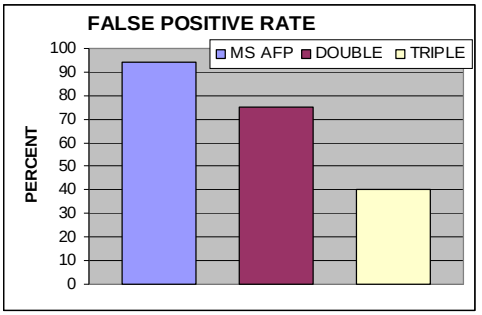

Figure-1: Graphical representation of the false positive rates for triple, double, MSAFP tests in relation to actual data after birth (MSAFP 94%, double test 75% and triple test 40%).

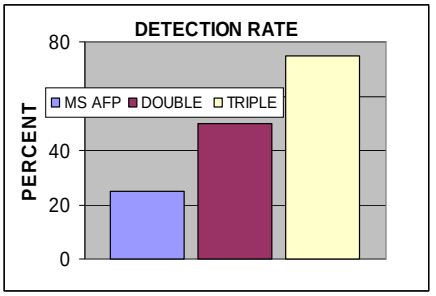

Figure-2: Graphical representation of the detection rates for triple, double, MSAFP tests in relation to actual data after birth (MSAFP 25%, double test 50% and triple test 75%).

Correlation factors between each test and actual data after birth in addition of p value are shown in the following tables:

Table-1: shows the correlation factor and p value between the triple test and actual data after birth.

The comparison pair	N	Correlation coefficient	P value
After birth & triple marker	50	0.626	0.727

In the case of triple marker test and the after birth the correlation coefficient is 0.626 which is fairly near +1. This is an indication of a relation between the actual values after birth and calculated triple marker value. The p value of the triple test is more than 0.05; indicate a non-significant difference between the expected and the measured variable, i.e. the triple test is a good predictor for Down syndrome.

Table-2: shows the correlation factor and p value between double test and actual data after birth.

The comparison pair	N	Correlation Coefficient	P value
Double test & after birth score	50	0.267	0.334

In the case of the double marker test and the after birth; the correlation coefficient is 0.267 which is very low in approaching +1. This means that there is a very fair relation between the after birth score and expected values calculated from the double test data (this value indicates that there are many factors to be added to the double test to correctly predict DS). But the p value of 0.334 is higher than 0.05 to

say that the two groups are not significantly different i.e. the double test can be used to the limit for limit screening of Down syndrome.

Table-3: shows the correlation factor and p value between MSAFP test and actual data after birth.

Pair of comparison	N	Correlation coefficient	P value
MSAFP & after birth data	50	0.146	3.44 E-5

In the case of MSAFP test and the after birth; the correlation coefficient is 0.146 which is very low indicating very low relevance for a relation between these values or there are many factors that must be add to the MSAFP before we can give a good prediction of DS. The very low p value 3.44 E-5 means that there is a significant difference between the two groups of comparison. This concludes that the MSAFP test is not a good test for predicting Down syndrome. Results after using a cut-off value of 1:190 or 1:380 instead of 1:270 are shown in the Table-4: A cut-off value of 1:380 raises the false positive rate of the tests with no improvement of the detection rate. On the other hand, a cut-off value of 1:190 results in the same detection rate with a lower false positive rate of the tests.

Table -4: The detection and false positive rates for each test in case of changing the cut-off value to 1:380 or 1:190.

Cut-off	Rate	Triple test	Double test	MSAFP
Cut-off 1:380	Detection Rate	75%	50%	50%
	FPR	70%	78%	91%
Cut-off 1:190	Detection Rate	75%	50%	0%
	FPR	25%	33%	100%

DISCUSSION

In the present study, we chose a cut-off 1:270 or less for high risk pregnancies as it is the used cut-off in most studies for pregnant women 35 years or older. The reason for choosing that cutoff value was based on the risk of complications from an amniocentesis procedure. If the mother's risk was less than 1 in 270 of having a child with Down syndrome, then the risk of amniocentesis was greater and the mother was called "high risk". Likewise, if the mother's results showed a risk greater than 1 in 270, the pregnancy was called "low risk" (11).

Recently, however, the American College of Obstetricians and Gynecologists (AC OG) have advocated not using the terms "high risk" and "low risk", but instead presenting the parents with the actual numerical risk value (11).

According to the final report of this study, four cases had actual affected babies with Down syndrome (cases: 20, 29, 41 and 44) and all of the other 46 cases had unaffected babies.

As regards the MSAFP test, it resulted in 24 high risk cases and 26 low risk cases for achieving a Down syndrome baby. Out of the 24 high risk cases, only one case had a baby with Down syndrome (no.41) and all other 23 cases had unaffected babies. Out of the 26 low risk cases, 3 cases of them had affected babies with Down syndrome (cases 20, 29, 44) whereas the other 23 had unaffected babies.

The correlation coefficient between MSAFP test and actual outcome of pregnancy is 0.146, which means that there is a very weak relation between the test and actual outcome of pregnancy. P value in this comparison is very low (>0.05) meaning that there is a significant difference between the test and outcome

of pregnancy. From these results, we can report that the detection rate with MSAFP and age was 25% (detected 1 Down syndrome case out of 4 cases). This finding is nearly in agreement with Mooney et al., (12), who reported that the detection rate of MSAFP and age is 28%.

We also reported that the false positive rate of the test is 96%. One explanation of such a high false positive rate may be attributed to calculating weeks of pregnancy by use of last menstrual period not through ultrasonography leading to inaccurate calculations. The second is the overlapping of AFP test results between affected and unaffected pregnancies. As regards the double test (β-HCG and MSAFP), it resulted in 9 high risk cases and 41 low risk cases for achieving a Down syndrome baby.

Out of the 9 high risk cases, two cases had a baby with Down syndrome (cases: 20 and 41) and all other 7 cases had unaffected babies. Out of the 41 low risk cases, 2 cases of them had affected babies with Down syndrome (cases 29 and 44) whereas the other 39 had unaffected babies.

The correlation coefficient between double test and the actual outcome of pregnancy is 0.267, which means that there is a weak relation between the test and actual outcome of pregnancy. P value in this comparison is >0.05 meaning that there is non-significant difference between the test and outcome of pregnancy.

From these results, we report that the detection rate of the double test was 50% (detected 2 Down syndrome cases out of 4 cases). This finding is in agreement with Mooney et al., (12), who reported that the detection rate of MSAFP and β-HCG is 56%. This finding is also in agreement with MacDonald et al., (13), who reported that the detection rate of MSAFP and β-HCG is 48%.

This finding somewhat disagrees with Audibert et al., (14), who reported that the detection rate of MSAFP and β-HCG is 60%, Kellner et al., (15), who reported that the detection rate of MSAFP and β-HCG is 60%, Harrison et al., (16), who reported that the detection rate of MSAFP and β-HCG is 63%, Chao et al., (17) who reported that the detection rate of MSAFP and β-HCG is 67%, Lam et al., (18), who reported that the detection rate of MSAFP and β-HCG is 69% .

From these results, we deduced that the addition of β-HCG to the maternal serum AFP doubles the detection rate of the biochemical screening at maternal age of 35 years or more. We also deduced that the false positive rate of the test is 77%. This may be explained as the same as MSAFP high false positive rate.

As regards the triple test, the test resulted in 5 high risk cases and 45 low risk cases for achieving a Down syndrome baby. Out of the 5 high risk cases, three cases had a baby with Down syndrome (cases: 20, 29 and 41) and the other 2 cases had unaffected babies. Out of the 45 low risk cases, one case had an affected baby with Down syndrome (case: 44) whereas the other 44 cases had unaffected babies. The correlation coefficient between the triple test and actual outcome of pregnancy is 0.626, which means that there is a strong relation between the test and actual outcome of pregnancy. Although this relation is nearer to +1 more than it is to zero but it also means that other factors or analytes may be needed to be considered in the screening program to raise the correlation coefficient more. P value in this comparison is 0.727 meaning that there is not a significant difference

between the test and outcome of pregnancy.

From these results, we report that the detection rate of triple test combination was 75% (detected 3 Down syndrome cases out of 4 cases). This finding is in agreement with several studies as Benn et al., (19), who reported that the detection rate of the triple marker is 75%, Xia et al., (20), who reported that the detection rate of the triple marker is 77.77%, Mac-Donald et al., (13), who reported that the detection rate of the triple marker is 77%.

This finding also disagrees somewhat with several studies which resulted in higher detection rate as Lesin et al., (21), who reported that the detection rate of the triple marker is 80%, Valerio et al., (22), who reported that the detection rate of the triple marker is 81%, Haddow James et al., (23), who reported that the detection rate of the triple marker is 89%, Cheng et al., (24), who reported that the detection rate of the triple marker is 89%.

This finding also disagrees with several studies,which resulted in a lower detection rate of the triple test as Summers et al., (25), who reported that the detection rate of the triple marker is 70.6%, Muller et al., (26), who reported that the detection rate of the triple marker is 70.8%.

From these results, we rely on the addition of uE3 as a marker for Down syndrome screening. It improved the detection rate form 50% achieved by the use of β-HCG and MSAFP to 75% achieved by the triple test. This finding is in agreement with MacDonald et al. (13), who reported that if uE3 was omitted, the detection rate decreased from 77 to 48%. In another study, Goodburn (27) reported that the exclusion of uE3 from the screening protocol would have reduced the detection rate for the same false-positive level.

This finding also disagrees with several conclusions, which recommended against using uE3 levels as a marker for prenatal Down syndrome screening as Loncar (28) and Reynolds (29), who reported that the addition of uE3 to the screening protocol has not consistently improved detection rates, possibly because of its high correlation with AFP. In addition, David 1996 (30) reported that the addition of uE3 lowered the detection rate of Down syndrome pregnancies with only a small and insignificant effect on the false-positive rate.

We also report that the false positive rate of the test is 40%. This may be explained as the same as MSAFP and double tests high false positive rate. Although we used a high risk population in this study, it is expected that the observed superiority of the combination screen would persist in a population of younger women. The development of a combined biometric and serum analyte screening algorithm for estimating individual odds could represent an advance in prenatal Down syndrome screening.

Changing the cut-off value of the test: The aim of such a change in the cut-off value of the test is to find the best risk to use cut-off, which is the one that gives the best balance of detection versus false positives.

Cut-off 1:380: Such a change will result in higher false positive rate of all screening tests (70%, 78% and 91% for MSAFP, double and triple tests respectively). This change in our study raised the detection rate of MSAFP (from 25% to 50%) but the detection rate of double and triple tests remained the same (50 and 75% respectively). In conclusion, we disagree with this change as it will cause higher false positive results and no effect on detection rate of triple test.

Cut-off 1:190: In our study, changing the cut-off value to be 1:190 resulted in a detection rate of 0%, 50% and 75% for MSAFP, double and triple tests respectively. This showed that no change in the detection rate in the case of double and triples tests occured. At the same time, this cut-off lowered the false positive rate to 25% and 33% in cases of triple and double tests respectively.

In conclusion, we deduced that 1:190 is the preferred cut-off value to be used. This agrees with some authors who reported that this change in particular will result in a lower false positive rate and keeping the same detection rate or at least proves a balance between the detection and false positive rates of the test (31, 32 and 33).

CONCLUSION

The preliminary results of this study indicate that the triple test is the test of choice as regards the double test or MSAFP test for screening of Down syndrome. It has the highest detection rate and lowest false positive rate.

The correlation coefficient is strongest between triple test and outcome of pregnancy (0.626). At the same time p value is 0.727. This means that triple test is the best as regards MSAFP and double test in screening of Down syndrome but at the same time other factors or analytes are needed to make this correlation coefficient nearer to +1. Use of a cut-off value 1:190 is recommended as it keeps the same detection rate and lowers the false positive rate of the test.

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