Med Discoveries
An open access journal of science and medicine

Article Type: Short Commentary
Volume: 2, Issue 11
Received: Oct 18, 2023
Accepted: Nov 15, 2023
Published Online: Nov 22, 2023

Biochemical Screening and Pregnancy Outcome after Assisted Reproduction

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Abstract

Women conceived using Artificial Reproduction Technology (ART) are more likely to have a high risk of Down's Syndrome (DS) compared with the women who conceived naturally. On comparison with naturally conceived pregnancies, ART pregnancies show significant differences in the levels of biochemical markers during first and second trimester screening. In the study of 628 ART pregnancies, risk of DS was found in 3.5% women. On evaluation of the outcome data, screening test has 98.3% of specificity and 100% of sensitivity. It is concluded that biochemical markers are potential indicators for the risk of DS in ART pregnancy.

Keywords: Maternal serum screening; ART pregnancy; Down's syndrome; Biochemical screening; Singleton pregnancy; Twin pregnancy.

Introduction

The Assisted Reproduction Technology (ART) has become a boon to those parents who had no chance of having their own child and are now the proud parents. Pregnancies conceived using this technology represent a group of high risk pregnancies, which carry a higher psychological and financial burden as compared to the spontaneous pregnancies [1]. Risk of having a child affected with Down's syndrome (DS/trisomy 21/T21) in women aged 35 yrs or more, chromosomal aberration, high multi-fetal pregnancies are generally noticed in such pregnancies. Interpretation based on the change in the levels of biochemical markers in first and second trimester results in high false positive rate in earlier studies [2-7]. In India, there is a lacuna in this area as valid data for singleton and twin pregnancies achieved by ART has not been fully investigated. Therefore, a systematic reliable study is required to screen ART pregnancies for DS with low false positive rate.

The present study aimed to analyze the biochemical markers (Alpha Feto Protein (AFP), Free b-HCG, PAPP-A, UE3 in ART and non-ART pregnancies to predict the risk for Down's syndrome in singleton and twin pregnancies. It was planned to validate the screening data with pregnancy outcome to prove the clinical significance.

Methodology

Subjects: In retrospective study, data of 628 cases of singleton and twin IVF pregnancy in age group 20-40 yrs handled at Sir Ganga Ram Hospital, New Delhi with high T21 risk was collected and analyzed. The data of first trimester PAPP-A, free β-HCG done at gestational age (GA) of 11-14 weeks (wks), and second trimester AFP, free β-HCG and UE3 with ultrasound details done at GA of 15-20 wks using time resolved immuno- fluorometry method (Delfia Xpress, Perkin Elmer) were collected. For comparative study, 800 age and gestation matched normal control (non IVF pregnancy) subjects' data without any history of chromosomal abnormality/fetal anomalies were also collected. In addition to this information, pregnancy outcome data of all these subjects was collected simultaneously.

Data analysis: Down's risk and age risk data were analyzed in screen negative/positive ART cases. Multiples of median (MoM) of various biochemical markers in addition to ultrasound marker NT in ART pregnancies was compared with the results in normal control subjects (non-ART pregnancies). Medians of all parameters and inter quartile range were determined to further analyze the results.

Citation: Verma J, Jhingan G, Thomas DC, Bijarnia-Mahay S, Puri RD. Biochemical Screening and Pregnancy Outcome after Assisted Reproduction. Med Discoveries. 2023; 2(11): 1091.

Statistical analysis: Statistical analysis of data was performed with SPSS software version 17.0. Mann-Whitney U-test, a non parametric statistical tool was used for comparing average performance in terms of medians of two groups. Two sided p value <0.05 was considered to reflect statistical significance and shown with the mark # in the tabular data (Table 2).

Pregnancy outcome: After delivery, outcome of pregnancy was recorded telephonically by an experienced clinician to validate the screening results for Down's syndrome (Table 2). All screen positive cases were confirmed by karyotyping/Fluorescence in-situ hybridization (FISH) analysis. The sensitivity and specificity of biochemical screening (1st and 2nd trimester) were calculated to exhibit the clinical significance of serum screening in ART pregnancies.

Results

Down's risk with age risk: In the present study, Down's risk is observed in 2.9% women (14/476) with singleton pregnancy and 4.7% women (11/235) with twin pregnancy. In both type of pregnancies, women of age group 30-39 yrs are found to be more prone to have Down's risk. Women with singleton pregnancy have Down's risk of 1:52 whereas, it is 1:140 in women with twin pregnancy. We have not found Down's risk in any women of age groups >40 yrs with singleton pregnancy and 20-29 yrs with twin pregnancy.

Deviation in MOMs of biochemical markers and NT singleton/twin pregnancy (Table 1): All screen positive cases in ART pregnancies showed Down's risk. In these cases, free β-HCG goes up significantly (up to 260% of the non-ART) in both trimester cases (first trimester-singleton MoM: 1.63 vs. 0.9, twin MoM: 1.54 vs. 1.2 and second trimester-singleton MoM: 3.96 vs. 1.2, twin MoM: 3.93 vs. 2.0).

PAPP-A MoM goes down about 64% of the normal (0.36 vs. 1.0) while significant increase in NT (up to 200%) is seen in first trimester (singleton/twin MoM: 2.4 vs. 0.8). The median values of MoMs of AFP (singleton MoM: 0.47 vs. 1.0, twin MoM: 1.4 vs. 1.85) and UE3 (singleton MoM: 0.75 vs. 1.34, twin MoM: 1.07 vs. 1.9) goes significantly down (24-53%) in second trimester. These statistically significant differences in the biomarkers as well as in NT are clear indications for fetal anomaly.

Table 1: Status of biomarkers and NT in ART pregnancies with Down's risk in comparison to normal pregnancies conceived naturally.

Pregnancy	Testing	Parameters				
		Free β HCG	PAPP -A	NT	AFP	UE3
Singleton pregnancy	First trimester	↑	↓	↑↑	ND	ND
	Second trimester	↑↑	ND	ND	↓	↓
Twin pregnancy	First trimester	↑	↓	↑↑	ND	ND
	Second trimester	↑↑	ND	ND	↓	↓

Foot note: ↑ - represents increase level. Numbers of arrows represent degree of increase in level.
↓ - represents decrease level. Numbers of arrows represent degree of decrease in level. ND- not done.

Pregnancy outcome (Table 2): Pregnancy outcome data of 682 cases is presented. Among them, 677 are found to be normal while 5 (3 singleton and 2 twin pregnancy cases) are affected with Down's syndrome as confirmed by karyotyping/FISH analysis. Ultrasound marker NT is almost 3.1 times higher in affected cases in comparison to unaffected population ($p<0.05$). Median values of MoMs calculated for various parameters with inter quartile range (IQR) presented in Table 2 shows that in singleton pregnancy, difference in the level of biochemical markers (free β-HCG (2nd trimester), PAPP-A, AFP and UE3) is more in singleton pregnancy than the difference in twin pregnancy in comparison to normal pregnancy conceived naturally.

Table 2: Pregnancy outcome (n=1482) results of the biochemical screening in ART and non-ART cases with performance evaluation.

Variables		Pregnancy outcome						
		Singleton - Non-ART	Singleton - ART		Twin - Non-ART	Twin - ART		
		Normal - unaffected (n=)	Unaffected (Normal) (n=458)	*Affected (DS) (n=18)	Normal - unaffected (n=)	Unaffected (Normal) (n=199)	*Affected (DS) (n=7)	
Maternal age (in yrs) Median (IQR)		34(23-45)	34(24-44)	31(29-35)	33 (23-45)	35 (26-45)	36(33-38)	Biochemical screening parameters
NT MoM, Median (IQR)		0.8(0.78-0.95)	0.79(0.69-0.92)	2.4#	0.8 (0.76-0.95)	0.77(0.69-0.95)	2.41#	
PAPP-A MoM,Median (IQR)		1.0(0.62-1.33)	1(0.58-1.52)	0.36#	1.0 (0.97-1.2)	2.35(1.57-3.30)	2.57	
Free β- HCG oM, Median (IQR)	1st trimester	0.9(0.67-1.87)	0.86(0.54-1.89)	1.63	1.2 (0.9-1.43)	1.51(1.00-2.43)	1.54	
	2nd trimester	1.1(0.68-1.89)	1.15(0.68-2.04)	3.96#	2.0 (1.43-2.75)	2.02(1.21-3.01)	3.93	
AFP MoM, Median (IQR)		1.0(0.83-1.49)	1.07(0.71-1.53)	0.47#	1.85 (1.51-2.45)	1.85(1.45-2.85)	1.8	
UE3 MoM, Median (IQR)		1.34(0.98-1.65)	1.34(1.02-1.75)	0.75#	1.9 (1.41-2.27)	1.9(1.38-2.34)	1.07	
Age risk		611(455-1011)	660(390-959)	1:551, 1:150, 1:60	725 (401-951)	664(460-920)	1:420, 1:85	
Down's risk		11000(3587.5-38700.2)	14000(3961.2-35752.5)	(1:190, 1:44, 1:15)#	9400(2100.4-18500.3)	8300(1880.5-19000)	(1:200, 1:200)#	

Karyotyping / FISH		Done in 5 FP cases	√		Done in 6 FP cases	√	Pregnancy outcome – Validation data
Down's syndrome		X	√		X	√	
FN, n (% rate)	Zero (0.0%)						
FP, n (% rate)	11/677x100= (1.6%)						
PPV	TP/TP+FPx100= 5/16 x100= 31.2%						
NPV	TN/ TN+FNx100= 666/666x100=100%						
Sensitivity	TP/TP+FNx100= 5/5x100=100%						
Specificity	TN/TN+FPx100=666/677x100=98.3%						
Accuracy	666+5/682x100= 98.3%						
Total error	11/682x100=1.6% (Whole study)						

Footnote: Abbreviations: DS: Down's Syndrome; NT: Nuchal Translucency; TP: True Positive; TN: True Negative; FP: False Positive; FN: False Negative; FISH: Fluorescence In-Situ Hybridization; N = Number; PPV: Positive Predictive Value; NPV: Negative Predictive Va1ue.
*Interquartile Range (IQR) is not available due to less numbers (n=<3) to calculate. #Two tailed p-value (<0.05) -significant.

Validation of screening data with outcome data: In the present study, there is no false negative while false positive rate is 1.6% (Table 2). Based on the outcome data, sensitivity and specificity of the biochemical screening tests (1st trimester and 2nd trimester) in ART cases are 100% and 98.3% respectively. The rigorous monitoring of intra and inter assay coefficient of variation within acceptable limits (<20%) for each of these assays ensured excellent testing quality.

Conclusion & clinical implications

Although amniocentesis and Chorionic Villi Samples (CVS) are recommended in cases at high risk of fetal anomaly, invasive procedures have been more challenging in ART pregnancies and women are usually reluctant to accept such testing due to the risk of miscarriage [8]. For all these reasons, non-invasive biochemical serum screening become favorable and it should be as accurate as possible in such pregnancies. Our study showed 98.3% accuracy with 100% sensitivity of biochemical screening in ART cases for Down's syndrome which is quite improved in comparison to the earlier reported data.

Since ART pregnancy differs from natural pregnancy in terms of growth and development of fetus and placenta, the deviation in biochemical markers could occur due to the delay in placental maturation. In addition, other factors like relationship with multiple corpora lutea, multiple implantation sites or drugs used have been reported to alter the metabolism in both fetus and placenta [9-11]. High false positive rate due to the drastic change in biochemical markers (e.g. decrease in PAPP-A level) has been unfavorable because of elevation in invasive testing with increasing risk of fetal loss [12]. Hence, careful interpretation with respect to the deviation in the MoMs of biochemical markers and ultrasound marker NT could reduce the false positive rate. From pregnancy outcome data, we found 1.6% of FPR while negative predictive value was 100%. Therefore, by applying correction factor for specific marker, accuracy of screening results for ART pregnancies study can be improved. The low total error rate (1.6%) in the present study proves the same. Markedly differences in biochemical markers in the pregnancies conceived by ART in comparison to normal pregnancies is a strong predictor of Down's risk and therefore, screening is highly recommended in ART cases.

Declarations

Acknowledgement: The authors express their gratitude to all clinicians, obstetricians and gynecologists for referring IVF cases for biochemical screening. The contribution of all patients, Scientific and technical staff (Dr. Papai Roy, Sandeepika, Azad Singh and Divya Tiwari) and statistician (Dr. R.L. Sapra) is highly acknowledged.

Declaration of potential conflict of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

Declaration for submitted article: The work described in this article has not been published previously. It is not under consideration for publication elsewhere. Its publication is approved by all authors.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

Informed consent: All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000 (5) of the World Medical Association. Informed consent was obtained from all patients/normal subjects for being included in the study.

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