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Original Article

Biochemistry Section

First Trimester Combined Aneuploidy Screening for Trisomy 21: A Three Years Retrospective Study

VARSHA BIRLA[1], FLAVIA ALMEIDA[2], ALAP CHRISTY[3], GURURAJ PURANIK[4], RAJ JATALE[5], KIRTI CHADHA[6]

ABSTRACT

Introduction: Prenatal testing also known as maternal screening is primarily performed to screen out most common chromosomal anomalies in the foetus using maternal blood. It provides an accurate and sensitive assessment of a patient's risk of carrying a foetus with chromosomal anomalies.

Aim: To study the first trimester prenatal screening using a Foetal Medicine Foundation (FMF) certified platform to estimate the risk of foetal trisomy 21.

Materials and Methods: This retrospective study was conducted at Global Reference Laboratory, Mumbai, Maharashtra, India, from January 2018 to March 2021 on samples of 86118 pregnant Asian women with respect to age, the risk cut-off was set at 1:250 for trisomy 21. The study included determination of free β-human Chorionic Gonadotropin (β-hCG) and Pregnancy Associated Plasma Protein-A (PAPP-A) in maternal serum, ultrasound studies of Crown Rump Length (CRL), Nuchal Translucency (NT) and nasal bone and maternal characteristics. Concentration of biochemical parameters was expressed in Multiple of Medians (MoM) respective to gestation age. Risk assessment of trisomy 21 was analysed using lifecycle software cut-off being 1:250 at sampling.

Results: The overall positive risk (high risk) for trisomy 21 obtained was 2.58%, association with advanced maternal age, history of Insulin dependent diabetes mellitus and absent nasal bone status. Biochemically, the MOM of β-hCG was high with mean MoM of 2.40 (>1.5) and MoM of PAPP-A was low with mean MoM of 0.64 (≤0.6).

Conclusion: This study enabled us to understand the importance of prenatal testing, a non invasive screening of chromosomal disorders like trisomy 21 which gives the advantages of early counseling and diagnosis quite as early as in the first trimester of pregnancy. It helps in drastically reducing the use of invasive procedures associated with risk of miscarriages. It is suggested that screening for chromosomal abnormalities be offered in all antenatal women irrespective of age and parity.

Keywords: Crown rump length, Human chorionic gonadotropin, Multiple of medians, Nasal bone, Nuchal translucency, Pregnancy associated plasma protein A

INTRODUCTION

Prenatal testing also known as maternal screening is primarily performed to screen out most common chromosomal anomalies in the foetus using maternal blood. It provides an accurate and sensitive assessment of a patient's risk of carrying a foetus with chromosomal anomalies [1]. Earlier the screening was offered to only elderly pregnancies however there are several studies and statistics proving the risk of trisomy in young age group as well. Thus, all pregnant females should be offered both screening and diagnostics tests irrespective of maternal age, all should undergo counselling and have the right to accept or reject the test. Screening inturn also helps in reducing the risk of miscarriage involved in undergoing invasive procedure [2].

First trimester combined screening involves Nuchal Translucency (NT) screening along with "dual marker" or double marker test. NT is the sonographic appearance of a collection of fluid under the skin behind the foetal neck in the first trimester of pregnancy. The term translucency is used, irrespective of whether it is septated or not and whether it is confined to the neck or envelopes the whole foetus. Because slight misinterpretations in evaluating NT values will cause wide variation in accuracy of the calculations, it is essential to combine NT with maternal serum screening and is the recognised first trimester screen performed between 10 and 13.6 weeks of gestation [1,3,4].

Screening tests are associated with identifying Patau, Edwards and Downs syndromes (trisomy 13, 18 and 21, respectively), and the less severe Turner (monosomy X) and Klinefelter (XXY) syndromes. First trimester screening means screening for trisomy's (13,18,21) and trisomy 21 being the most common cause of mental retardation is included in this screening programme [5]. Downs syndrome, with an incidence rate of 1 in 800 pregnancies, is the predominant reason for women seeking prenatal diagnosis [6,7].

The invasive diagnostics test like amniocentesis and chorionic villous sampling required for karyotyping and confirmation of trisomy's carries risk of miscarriage and currently these procedures are offered to only a small group of pregnant women who are at high risk of having an offspring with a chromosomal defect as compared to the general population [8]. The aim of the currently available screening tests is actually to identify, with the highest possible sensitivity and specificity, those women who should be offered the invasive procedure. The risk for many of the chromosomal defects increases with maternal age. Additionally, because foetuses with chromosomal defects are more likely to die in-utero than normal foetuses, the risk decreases with gestational age.

Objective of this study was to understand positivity amongst different age groups with the variables such as clinical history, type of pregnancy and Ultrasound (USG) markers. This would help to target the high-risk population at an early stage.

MATERIALS AND METHODS

This retrospective study was conducted at Global Reference Laboratory, Mumbai, Maharashtra, India, from January 2018 to March 2021, included samples of 86118 pregnant Asian women with respect to age, the risk cut-off was set at 1:250 for trisomy 21 [4]. The maternal age ranging from 18 years to 50 years of age was considered. Biochemical markers like free β-human Chorionic Gonadotropin (β-hCG) and Pregnancy Associated Plasma Protein-A (PAPP-A) was performed using maternal blood from 11 to 13.6 weeks of gestation on PerkinElmer® platform by Time Resolved Fluroimmunoassay

(TRF). Information about the patient's age, clinical history details were collected and taken from the maternal Test Requisition Form (TRF) and Ultrasonography report. First trimester combined screening was performed by calculating risk using software with ultrasound findings, serum biochemical markers and maternal characteristics [9]. The study was conducted retrospectively from the data available in information system of the laboratory. The approval was obtained to use this data for publication from Conscience Independent Ethics Committee (wide letter dated 2nd June 2021).

Assay

Maternal serum concentrations of Pregnancy Associated Plasma Protein-A (PAPP-A) and free β-hCG biomarkers have been read with PerkinElmer® kits {Foetal Medicine Foundation (FMF) certified} on AutoDELPHIA platform operates on the principle of time resolved fluroimmunoassay. Maternal characteristics like age, weight, ethnicity, history of smoking, diabetes, previous history of trisomy, gestation of foetus, number of foetus, mode of conception was taken into consideration. Ultrasonography details like date of USG, Crown Rump Length (CRL), Nuchal Translucency (NT) and nasal bone status was incorporated. The processing of data and determination of the risk of trisomy 21 has been done with LifeCycle 7.0 software (Perkin Elmer Prenatal software).

The measured concentration of free β-hCG and PAPP-A is converted into MoM appropriate to the gestation age of pregnancy [2]. The Multiples of the Median (MoM) value is obtained by dividing an individual's marker concentration by the median level of that marker for the entire population at the same gestational age in that laboratory [10].

STATISTICAL ANALYSIS

Data was analysed using R software version 3.5.2. Result of quantitative variable like β-hCG MoM, PAPP-AMoM, NT MoM, trisomy 21 age risk are expressed as mean+Standard Deviation (SD), median {Interquartile Range (IQR)} and range. Result of qualitative variable like age at Expected Date of Delivery (EDD) (in years), weight (in kg), gestational age (in weeks), number of foetuses, smoking history, ethnicity, assistance method, diabetic, nasal bone, trisomy 21 is expressed in number and percentage. Shapiro-Wilks Test was used to determine whether data sets differed from a normal distribution. For categorised variables like age group, gestational age, weight, smoking, ethnicity, nasal bone compared to trisomy 21 Pearson's Chi-square test or Fisher's-exact test has been used. For continuous variable between two groups was compared using Mann-Whitney U test and for three or more groups Kruskal-Wallis test was used based on normality testing. To determine the independent effects of variables associated with the positive trisomy 21, a multiple binary logistic regression analysis was then performed including variables with a p-value <0.1 from bivariate analysis. Result was considered significant at p-value <0.05.

RESULTS

Dual marker maternal screening was performed on FMF certified platform and a total of 86118 cases were studied from January 2018 to March 2021.

Total 72.2% of pregnant females were in the age group of 26-35 years. A significant number of pregnant females lies in elderly age group of ≥36 years (12.45%) [Table/Fig-1]. The overall positive risk (high risk) for trisomy 21 obtained was 2.58% [Table/Fig-2]. Cases mentioned under the category of unknown were excluded from statistical calculation (unknown are those cases which failed to provide specific demographic data).

There was significant association of risk for Downs syndrome with increased maternal age [Table/Fig-3]. Risk of trisomy 21 was found to be as high as 6.11% in maternal age group of 36 years to 40 years. Association was seen with presence of maternal history of insulin dependent diabetes [Table/Fig-3].

Variables	Number	Percentage
Age at EDD (in years)		
≤20	716	0.83%
21-25	12437	14.44%
26-30	33558	38.97%
31-35	28684	33.31%
36-40	9542	11.08%
>40	1181	1.37%
Weight (in kg)		
≤45	7161	8.32%
46-65	52151	60.56%
66-85	21228	24.65%
>85	2427	2.82%
Unknown	3151	3.65%
Gestational age (in weeks)		
11-11.6	10218	11.87%
12-12.6	40248	46.73%
13-13.6	35652	41.40%
Number of foetus		
1	84109	97.67%
2	2009	2.33%
Smoking		
No	85626	99.43%
Yes	34	0.04%
Unknown	458	0.53%
Ethnicity		
Afro-Caribbean	239	0.28%
Asian	85585	99.38%
Caucasian	225	0.26%
Other	67	0.08%
Assistance method		
Donor egg	473	0.55%
Donor insemination	13	0.02%
Intra-Cytoplasmic Sperm Injection (ICSI)	262	0.30%
In-vitro fertilisation	1243	1.44%
Other	1	0.01%
Natural conception	84126	97.69%
Diabetic		
No	85268	99.01%
Yes	205	0.24%
Unknown	645	0.75
Nasal bone		
Absent	366	0.42%
Present	80223	93.15%
Unknown	5539	6.43%
Previous history of trisomy 21		
No	78225	90%
Yes	77	1%
Unknown	7816	9%

[Table/Fig-1]: Frequency and percentage of maternal screening cases with respect to age, weight, gestational age, foetus, race, smoking, diabetes status, nasal bone and assistance method.

Trisomy 21 screen	Number	Percentage (%)
Negative	83893	97.42
Positive	2225	2.58

[Table/Fig-2]: Trisomy 21 Screen positive and negative percentage.

Variables	Trisomy 21 screening (T21)				p-value
	Negative		Positive		
	Frequency	Percentage	Frequency	Percentage	
Age at expected date of delivery (years)					
≤20	703	98.18	13	1.82	**0.0001**
21-25	12225	98.30	212	1.70	
26-30	33000	98.34	558	1.66	
31-35	27991	97.58	693	2.42	
36-40	8959	93.89	583	6.11	
>40	1015	85.94	166	14.06	
Weight (kg)					
≤45	7023	98.07	138	1.93	**0.0001**
46-65	50868	97.54	1283	2.46	
66-85	20609	97.08	619	2.92	
>85	2366	97.49	61	2.51	
History of insulin dependent diabetes mellitus					
No	83066	97.42	2202	2.58	**0.0007**
Yes	192	93.66	13	6.34	
History of smoking					
No	83409	97.41	2217	2.59	0.2268
Yes	32	94.12	2	5.88	
Assistance method					
Natural conception	81963	97.43	2163	2.57	0.1323
Assisted reproduction	1930	96.89	62	3.11	

[Table/Fig-3]: Maternal characteristics.
p-value <0.05 was considered significant; Unknown cases for these parameters have been excluded from statistical analysis

The risk of T21 was high in cases with absent (unossified/hypomineralised) nasal bone [Table/Fig-4]. Although 69.67% of foetus with absent nasal bones were normal foetuses, still it is one of the soft markers in the risk assessment of aneuploidy.

Biochemically, the MOM of β-hCG was high with mean MoM of 2.40 (>1.5) and MoM of PAPP-A was low with mean MoM of 0.64 (≤0.6) [Table/Fig-5].

Nasal bone	Trisomy 21 screening (T21)				p-value
	Negative n (%)		Positive n (%)		
Absent	255	69.67	111	30.33	**0.0001**
Present	78515	97.87	1708	2.13	

[Table/Fig-4]: Significance of Nasal bone status (in foetus ultrasound).
Unknown cases for these parameters have been excluded from statistical analysis
p-value <0.05 considered significant

Variables		N	Mean±SD	Median (IQR)	Range	p-value
β-hCG MoM						
Trisomy 21	Negative	84130	1.1451±0.8196	0.94 (0.64-1.40)	0-20.55	**0.00001**
	Positive	1988	2.4075±1.7662	2.025 (1.28-3.06)	0.16-22.49	
PAPP-A MoM						
Trisomy 21	Negative	84331	1.1456±0.6782	1 (0.68-1.45)	0-22.83	**0.00001**
	Positive	1987	0.6441±0.4839	0.51 (0.31-0.83)	0-4.76	
NT MoM						
Trisomy 21	Negative	83893	1.0007±0.3035	0.97 (0.81-1.15)	0.04-11.91	**0.00001**
	Positive	2225	1.5152±0.8657	1.29 (0.97-1.86)	0.30-9.77	

[Table/Fig-5]: MoM of biochemical parameters and Nuchal Translucency (NT) amongst screen positive and negative cases.
p-value <0.05 considered significant

The age-related risk for Trisomy 21 increases with increase in maternal age [Table/Fig-6].

Age (years)	Trisomy 21 screen (T21) age risk				p-value
	N	Mean±SD	Median (IQR)	Range	
≤20	716	1203.08±269.24	1024(990-1544)	887-1562	**0.0001**
21-25	12437	1126.20±257.43	97 (921-1427)	8-1537	
26-30	33558	908.63±228.37	829 (724-1109)	118-1380	
31-35	28684	553.12±174.87	529 (428-631)	87-965	
36-40	9542	227.37±83.59	218 (164-270)	62-428	
>40	1181	66.18±27.51	65 (48-80)	5-129	

[Table/Fig-6]: Age risk mean for Trisomy 21.
p-value <0.05 considered significant

Multivariate analysis identified patient with age group >40 years (p-value=0.0001, OR=7.5533), 36-40 year (p-value=0.0007, OR=2.8636), gestational age 13-13.6 weeks (p-value=0.0416, OR=1.1783), NB (p-value=0.0001, OR=1.6295), Ethnicity Caucasian (p-value=0.0001, OR=3.623), β-hCG MoM (p-value=0.0001, OR=2.7094, NT MOM (p-value=0.0001, OR=9.6457) where independently associated with positive T21 [Table/Fig-7].

Parameters	Multivariate analysis		
	p-value	OR	95% CI OR
Age group (years)			
≤20	Ref		
21-25	0.1287		
26-30	0.272		
31-35	0.8722		
36-40	0.0007	2.8636	1.5616-1.9240
>40	0.0001	7.5533	4.0329-14.1466
Weight (kg)			
≤45	Ref		
46-65	0.0771		
66-85	0.3678		
>85	0.6684		
Gestational age (in weeks)			
11-11.6	Ref		
12-12.6	0.0019	0.7727	0.6568-0.9092
13-13.6	0.0416	1.1783	1.0063-1.3797
Number of children			
1	Ref		
2	0.0174	0.0482	0.0377-0.0617
Nasal bone			
1	Ref		
2	0.0001	1.6295	0.6532-1.9218
Ethnicity			
Asian	Ref		
Afro-Caribbean	0.4999		
Caucasian	0.0001	3.623	1.9194-6.8418
Other	0.5329		
Diabetic			
No	Ref		
Yes	0.1486		
β-hCG MoM	0.0001	2.7094	2.5905-2.8338
PAPP-A MoM	0.0001	0.0351	0.0289-0.0425
NT MoM	0.0001	9.6457	8.4273-11.0402

[Table/Fig-7]: Multivariate analysis.
**OR: Odds ratio; Ref: Reference; p-value <0.05 considered significant

DISCUSSION

A screening programme should be efficient enough to identify an anomaly at right time and should be able to offer a solution at the same time. Now-a-days, prenatal screening and diagnosis between 10 to 14 weeks of gestation is becoming more and more available and efficient worldwide. Recent studies by Malone FD et al., also concluded that the sensitivity of first trimester screening (87%) is much more than second trimester tests (81%) [3,11]. So apart from the fact of higher sensitivity in comparison to other tests the first trimester screening method is also cost-effective since the indication of number of invasive procedures will be clearly defined.

One of the biggest advantages of the first trimester screening test is that counselling and invasive diagnosis of chromosomal defects can be accomplished early in pregnancy thus significantly reducing the anxiety. By providing the option of early termination of pregnancy the situation becomes less complicated for both the patient and the obstetrician [12]. According to Carlson LM and Vora NL, one should remember that the double marker test is only a screening test which provides a risk for the genetic disorder, but not the diagnosis [13].

The outcome of prenatal screening is in the form of screen positive or screen negative for Trisomy 21 and these results are based on the laboratory specific cut-offs [14]. The sensitivity of the estimated risk significantly depends on the accuracy of the information provided. The necessary adjustments are made in the measured maternal serum concentration of free β-hCG and PAPP-A in accordance with the gestational age, maternal age, weight, ethnicity, smoking status, history of insulin dependent diabetes mellitus, method of conception, parity [15].

Hence, it is essential to submit accurate information along with maternal sample to laboratory for risk assessment failing which may lead to significant alterations. In the present retrospective study, the risk evaluation was calculated at sampling and there was statistically significant increase in risk for Downs syndrome in pregnant females with history of insulin dependent diabetes mellitus, twin gestation and absent/unossified nasal bone status. Although there was no statistically significant increase in risk with respect to history of smoking and assisted reproduction, but the risk was high in cases with history of smoking and cases of In-Vitro Fertilisation (IVF) pregnancies in accordance with Hook EB and Cross PK book- Cigarette Smoking and Downs Syndrome [16,17]. Previous studies were compared with the present study [Table/Fig-8] [2,12,14,16,18].

Trisomy 21 risk			
Author	**31-35 years**	**36-40 years**	**>40 years**
Carlson LM et al., [11]	1:352	1:85	1:35
Shiefa S et al.,[12]	1:249	1:62	1:16
Ziolkowska K et al.,[2]	1:31.2	1:68.8	NA
Zournatzi V et al.,[10]	1:348	1:99	1:24
Snijders R and Nicolaides K [17]	1:249	1:60	1:38
Present study (2022)	1:553	1:227	1:66

[Table/Fig-8]: T21 Age Risk comparison amongst various studies [2,10,12,16,18].

In the present study, it was demonstrated that the age-related risk for Trisomy 21 increases significantly with maternal age. Mean estimated Risk of 1:1200 for age group less than 20 years to 1:66 in maternal age greater than 40 years. This can be used as an independent marker in interpreting trisomy 21 age related risk. This was in accordance with review article published by Shiefa S et al., in 2013 [14]. A molecular biology study has demonstrated that in trisomy 21 there is marked increase in free β-hCG concentration. Decreased levels of PAPP-A are found in association with abnormal placental function which has formed the basis for the first trimester screening of foetal Downs syndrome [19].

In the current study, the biochemical marker behaved in the similar fashion strengthening the above association. Using MoM values, rather than absolute levels, also allows results from different laboratories to be interpreted in a consistent way. In euploid pregnancies, the average adjusted value for both free β-hCG and PAPP-A is 1.0 MoM at all gestations [14]. In the present study, the MoM of Trisomy 21 high risk cases free β-hCG was high with mean MoM of 2 (>1.5) and MoM of PAPP-A was low with mean MoM of 0.6 (≤0.6) [Table/Fig-5].

The strength of the present study includes the huge sample size with varied demographic findings, evaluation using a FMF certified platform.

Limitation(s)

The limitation of this study was that the data of outcome analysis with respect to confirmatory testing was not available to understand the true positive rate. An insignificant number of cases (9%) failed to provide accurate demographic data like previous history of trisomy, history of smoking, diabetes mellitus and nasal bone status. The present study was based on large number of data points in Indian population which gives a better scenario of Indian Subcontinental clinical picture.

CONCLUSION(S)

The risk of foetal chromosomal anomaly like trisomy 21 is not limited to elderly women and thus, the prenatal first trimester screening should be offered to all pregnant females irrespective of maternal age. The dual marker screening performed in first trimester have high sensitivity, so abnormalities can be screened in early gestation and provides enough time to perform confirmatory testing and for decision making. It should be kept in mind that screening tests are not diagnostic but they can indeed alter the odds.

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PARTICULARS OF CONTRIBUTORS:
1. Clinical Biochemist, Department of Clinical Chemistry, Metropolis Healthcare Ltd., Mumbai, Maharashtra, India.
2. Senior Manager, Department of Clinical Chemistry, Metropolis Healthcare Ltd., Mumbai, Maharashtra, India.
3. Head, Department of Clinical Chemistry, Metropolis Healthcare Ltd., Mumbai, Maharashtra, India.
4. Doctor Incharge, Department of Clinical Chemistry, Metropolis Healthcare Ltd., Mumbai, Maharashtra, India.
5. Biostatistician, Department of Medical Affairs, Metropolis Healthcare Ltd., Mumbai, Maharashtra, India.
6. Chief of Scientific Affairs, Department of Medical Affairs, Metropolis Healthcare Ltd., Mumbai, Maharashtra, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Varsha Birla,
Kohinoor City, Commercial Building-A, 4th Floor, Off-L.B.S. Marg,
Vidyavihar (West), Mumbai-400070, Maharashtra, India.
E-mail: Varsha.birla@metropolisindia.com

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