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Investigating the presence of fetal trisomy 13, 18, and 21 in Pakistani Patients and its' Computational Analysis.

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Abstract

Background: Chromosomal aneuploidy due to meiotic non-disjunction is among the most significant and common causes of miscarriages in humans. The present study was conducted to investigate the presence of fetal trisomy 13, 18, and 21 in Pakistani patients and analysis of its risk factors.

Methodology: Fetuses were diagnosed for trisomies between 10 and 16 weeks of gestational age via Chorionic Villus Sampling. Fetal DNA was extracted through the Chelex method. The presence of trisomies 13, 18, and 21 was screened through amplification of Short Tandem Repeats (STR) on chromosomes 13, 18, and 21, respectively. The STR alleles were resolved through polyacrylamide gel electrophoresis (PAGE) and silver staining.

Results: It was found that out of 367 patients tested between 2012 and 2018, 3 (0.82%) had trisomy 13, 3 (0.82%) had trisomy 18, and 34 (9.3%) had trisomy 21. Down syndrome (Trisomy 21) was the most common trisomy in Pakistani patients as compared to 13 and 18 trisomies. Via R analysis, it is assessed that cousin marriages and mother's age contribute positively towards trisomy; nevertheless, the presence of these factors does not confirm the presence of trisomy for any chromosome.

Conclusion: It was observed that the age of the mother, delayed conception, and family marriages are the contributory factors for this problem. However, the prevalence of Trisomy 21 (Down syndrome) in the Pakistani Population is far greater as compared to Trisomy 13 (Patau syndrome) and Trisomy 18 (Edward syndrome).

Keywords

Chorionic Villus Sampling, Trisomies, Polyacrylamide Gel Electrophoresis.

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Introduction

Most of the babies suffering from Trisomy 13, 18, and 21 lose their lives at a very early age[1]. The increasing incidence of autosomal trisomies (13, 18, and 21) in newborns has both individual and socioeconomic consequences[2]. In the later stages of pregnancy, it is difficult to terminate the pregnancy due to complications and religious beliefs, so it is important to have a diagnosis scan at the early stages. In Pakistan, a lower rate of termination of pregnancy (TOP) for the congenital anomaly was reported[3]. In Punjab, Pakistan, the prevalence of congenital heart disease (Down syndrome) was 41.8%[4]. The Prevalence of Down Syndrome in the Pakistani Population was 16 out of 10,000 live births[5,6].

The occurrence of trisomy depends on the age of the mother, and it can differ among populations[7]. According to World Health Organization, approximately 3000 to 5000 children are born with Trisomy 21 annually[8]. Trisomy 18 (Edwards syndrome) is the world's second most common autosomal trisomy in newborns, with a prevalence of 1/7000 births[9]. For the characterization of multiple types of DNA markers used for this study, a total of seven studies have been published so far[10]. From these studies, it was determined that among 176 cases, 91% of cases had a maternal origin, and 9% had a paternal origin.

Most of the newborns with trisomy 13 and 18 die in the first year. Those who survive have serious medical complications. A group of people believes that it is useless to try to prolong the lives of such babies only to cause them more pain, but most other people are of the opinion that every chance should be taken to prolong the life span of such babies[11]. A study was conducted, and it was found that the children suffering from trisomy (13, 18, and 21) have improved survival rates when given proper aggressive medical intervention[12]. Two medical attitudes towards children with trisomy are palliative care or life-prolonging interventions[13]. Coordination of biomedical evaluations, psychosocial assessments, and care delivery models are important for children with trisomy 13 and 18[14].

The parental origin of Trisomy13 was determined in a study of 42 cases, showing 88% maternal errors and 12% paternal errors[15].

Detection of such fetal chromosomal aberrations is the first step towards their eradication. Chorionic Villus Sampling (CVS) combined with STR analysis provides a very promising solution for the detection of trisomy.

Methodology

The analytical research was done including age, gender, mother's age, and demography of subjects involved in the study and the fetal sampling for the pre-natal tests using CVS at 13 weeks of pregnancy. A total of 367 patients had been tested for trisomies from 2012-18. Blood samples of the parents and a previously affected child (if available) were also taken for testing.

DNA extraction was performed by washing 100 µl of whole blood with 1 ml distilled water and centrifuging at 5000 rpm for 1-2 minutes. The washing was repeated thrice, and 200 µl of 5% chelex was added to the precipitated pellet. The mixture was incubated at 95°C for 15 minutes and centrifuged at 13000 rpm for 2 minutes to obtain DNA as the supernatant. For the fetal sample, the washing was skipped, and 5% chelex was directly added to the fetal tissue to obtain DNA.

Short Tandem Repeat (STR) analysis was done for the detection of trisomy 13, 18, and 21. Four STR markers at most were used for each chromosome; D13S317, D13S258, D13S631, D13S634 for trisomy 13, D18S51, D18S386, D18S535, D18MBP for trisomy 18, and D21S11, D21S1411, D21S1414, D21S1412 for trisomy 21.

The PCR amplified products were run on 6% polyacrylamide gel and visualized after silver staining. Silver staining was carried out by submerging the gel in 0.2% silver nitrate solution ($AgNO_3$) for 15 minutes, then rinsing it with distilled water and dipping it in a tray with NaOH/formaldehyde solution (70 µl formaldehyde + 100 ml of 1.5% NaOH). After the bands were

visible, the gel was rinsed 2-3 times with tap water, placed on filter paper, and dried.

Lastly, the results of patients were analyzed via R language. Graphs were plotted (Dot-plots and Box-plots) to represent the relation of maternal age with the occurrence of this syndrome. The median of the plot depicts that the majority of the women that appeared for pre-natal diagnosis had their ages between 30 to 35 years, and the risk and prevalence of trisomies increase considerably in the case of 1st cousins. Dot plots represent the prevalence of trisomy 13, 18, and 21 in the Pakistani Population.

Results

From the genetic analysis, CVS diagnosis at Genetic Resource Centre (GRC Labs), and computational analysis via R language, it is estimated that the percentage of Patau syndrome in Pakistan is 0.78125%, Edward's syndrome is 0.833%, and Down syndrome is 9.014085%. After the collection of demographic data of patients, results were analyzed on the basis of ethnic groups, gestational age, mother's age, and consanguinity of parents. The ethnic groups observed were Punjabi, Sindhi, Baluch, Pathan, Saraiki, Hindko, Potohari, Afghani, Kashmiri, and Urdu, Speaking with the highest number of patients observed in Punjab (Table 1).

Table 1: Ethnicity of individuals tested for Trisomy 13, 18 and 21

Ethnicity	Frequency	Percentage
Punjabi	192	52.31%
Pathan	72	19.61%
Urdu	23	6.26%
Hindko	1	0.27%
Kashmiri	6	1.63%
Saraiki	2	0.54%
Baloch	1	0.27%
Hindu	1	0.27%
Afghani	1	0.27%
Unknown	68	18.52%

Most of the patients had their CVS done at 13 weeks of the gestation period, but some of them also had gone through this test in later weeks.

The results of silver-stained gel electrophoresis confirmed the presence of chromosomal aneuploidy in patients. Figure 1 (a, b, c & d) shows different results for the individuals tested for the presence of Down syndrome.

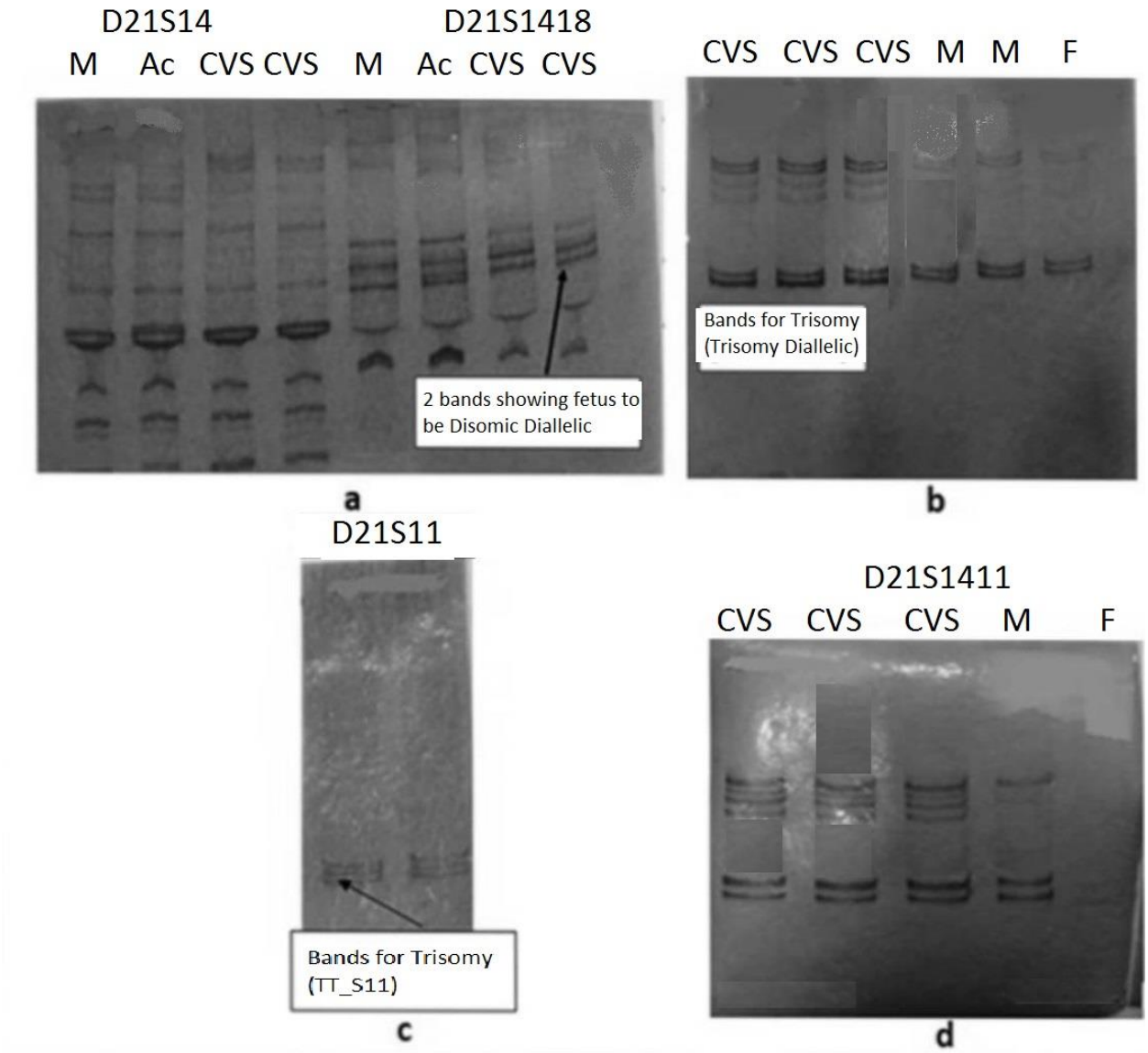

Figure 1: Silver-stained polyacrylamide gel electrophoresis results obtained for Trisomy 21 patients. Figure 1(a) shows the fetal chromosome 21 to be Disomic Diallelic with both S11 and S1411 as important marker sites. Figure 1(b and c) shows the fetal chromosome 21 to be Trisomic Diallelic and Trisomic Triallelic, respectively. Figure 1(d) depicts the fetal chromosome to be Trisomic Diallelic with S1411 to be an important marker site.

Out of the total 367 patients analyzed, total 32 individual fetuses were diagnosed with Down Syndrome with 25 mothers more than 35 years of age, and the two women with fetuses having Edwards syndrome and Patau syndrome were at the age of 41 and 38 years, respectively (Table 2). Out of 32 Down syndrome fetuses, only 8 had their parents unrelated, while the rest of the couples were either first cousins or belonged to the same family tree. Similarly, the parents of Trisomy 13 and 18 fetuses were also related. From Table 1, it can be seen that there are two ladies with just 26 years of age who are expecting fetuses with Trisomy 21; whereas there is a number of ladies with more than 40 years of age but still had normal fetuses.

Table 2: Table of Patients showing mother's age and consanguinity of parents for Trisomy 21 (Down syndrome), Trisomy 18 (Edwards syndrome), and for Trisomy 13 (Patau syndrome).

Variables		Frequency
Ages of mothers with high inbreeding coefficient (1st cousin)	30-39	3 (Trisomy 21)
	40-45	6 (Trisomy 21)
Ages of mother and frequency with related parents	20-29	2 (Trisomy 21)
	30-39	10 (1+1 case-trisomy 13, 18)
	40-45	5 (Trisomy 21)
Ages of mother and frequency with unrelated parents	30-39	6 (Trisomy 21)
	40-45	2 (Trisomy 21)

When the results were checked using D21 as a marker, it was found that 32 patients were trisomic, among which 6 were trisomic triallelic, and 26 were trisomic diallelic. 323 patients were disomic diallelic for D21. Eight patients showed monoallelic band and four patients were not tested with this marker.

It was found that the number of patients using D18 marker with disomic diallelic was 119. Only 1 patient was found to be having trisomic diallelic, and none of the patients showed triallelic trisomy when tested with D18 marker. Twenty eight patients showed to have a monoallelic band.

The results for the D13 marker showed that 127 patients were disomic diallelic, one patient was trisomicdiallelic, none of the patients was trisomic triallelic and 20 were identified as monoallelic.

Percentage of Trisomy 13, 18 and 21

32 patients out of 355 showed trisomy 21, which makes 9.01% of the total patients tested for trisomy 21. Only 1 patient among 120 had 3 bands for trisomy 18 and made 0.83% of the patients tested for trisomy 18. A total of 128 patients were tested for trisomy 13 from which 1 patient showed 3 bands having trisomy 13 which made 0.78% of the total patients tested for trisomy 13.

Discussion

The main objective of current study was to know the prevalence of three significant types of trisomies, i.e. Trisomy 13 (Patau syndrome), Trisomy 18 (Edwards syndrome), and Trisomy 21 (Down syndrome) in the Pakistani Population and their analysis. The most common chromosomal aneuploidy is trisomy 21, followed by trisomy 18, which is the second most occurring, and trisomy 13, which is the third most common cause of stillbirth. We found the highest prevalence of Down syndrome in the Punjabi Population, and the highest contributing factor was inbreeding confirming previous studies[16-18].

For a Long time, trisomy 13 has been having a poor prognosis and is associated with a wide range of congenital anomalies[19,20]. It is controversial to treat infants with trisomy 13 which is because of the deathly prognosis[21]. However, many medical and surgical treatments are evolving for the treatment of trisomy 13 and 18, including cardiac procedures[22-26]. Also, Trisomy 13 is also demonstrated to be associated with acute leukemia[27]. In our study, it was seen that parents of fetuses with trisomy 13 were related to each other. The number of cases studied is still very small; meiotic division error has not been established with certainty due to the lack of a useful centromere polymorphism and with considerable distance from the centromere to the most proximal long arm marker, and the issue of altered recombination has not been addressed. Anatomical and histopathological findings in 12 cases of trisomy 13 syndrome (9 with classic full trisomy and 3 with trisomy 13 and an unbalanced Robertsonian 13/13 translocation) are reported. Emphasis is on brain defects, cardiovascular anomalies, and histological organ dysplasia. Eight patients showed abnormal development of the forebrain and midline facial structures (holoprosencephaly). Cardiovascular

malformations were invariably present, the leading malformation being an infundibular ventricular septal defect often in combination with dextroposition of the aorta and abnormalities of the semilunar valves. Histological abnormalities giving evidence of organ dysplasia were observed in the central nervous system, eyes, pancreas, kidneys, and ovaries. Mild cystic renal dysplasia was a constant feature. Foci of persistent nodular renal blastema were found in six cases. The pancreatic dysplasia appears to be pathognomonic for trisomy 13. These observations illustrate the importance of pathological studies in recognition of chromosome abnormalities and, more specifically, of trisomy 13 syndrome. Based on autopsy data, trisomy 13 can be diagnosed - or ruled out - with certainty, even in the absence of karyotyping.

Different programming languages are used by researchers to keep the record of data and to analyze the data of research. The research was conducted on the children having trisomy 21, and the data was interpreted by using JAVA as a programming language[28]. We use the R language for the representation of our data. R is a system for statistical computation and graphics. It provides, among other things, a programming language, high-level graphics, interfaces to other languages, and debugging facilities[29]. We use R language to represent our results for easy understanding and presentation. Dot plots and box plots were used to express the results of the data for diagnosis of patients having trisomy 13, 18, and 21. Separate dot plots were made for each trisomy, but combined graphs are represented in this article to avoid any confusion.

From our data, it was found that Down syndrome (Trisomy 21) was the most occurring among 367 Pakistani patients in comparison to Trisomy 13 and 18. It was also observed that the greater the age of the mother, the more there is a chance of an anomaly of chromosome to occur. Cousin marriage was found to be an important risk factor enhancing the risk of having trisomy for the baby. These findings not only provide a comprehensive basis for carrying out CVS for Trisomies (13, 18, and 21) but also explain its pattern of prevalence in the Pakistani Population.

Pre-natal diagnosis using CVS before/13 weeks of gestation period provides a very promising solution for proper diagnosis. The problem in the Pakistani Population is the lack of awareness about the problem and how to deal with it. Certain labs like GRC (Genetic resource Center) lab are providing cost-effective techniques for pre-natal diagnosis. We should try to make this expensive test cost-effective for the general Population. Also, there is a need to make people aware of the consequences of cousin marriages if there is a prevailing disorder running in the family history because, in many cases, cousin marriages and the age of the mother were contributing factors. Awareness campaigns should be held for trisomies as it is not commonly known by the general public of Pakistan.

More future work should be done to answer the reason behind the influence of maternal age on chromosomal non-disjunction. To avoid all pre-natal procedures extracting the CVS fluid, a new T21 marker should be designed with high sensitivity and specificity[30,31].

Conclusion

From these results, we can conclude that although all above mentioned chromosomal abnormalities are not much in Pakistan still, there is a considerable need to eradicate them. The risk of these trisomies is directly proportional to the mother's age and consanguinity of parents. Another point worth mentioning is the low frequency of band visibility (via gel electrophoresis) at D21-S1412, D21-S1414, D18-S386, D18-S535, D18-MBP D13-S258, D13-S631, and D13-S634 chromosome marker sites does not mean that they are less informative. This is just because they are not tested very frequently. To conclude specifically, it was found that trisomies prevail in Pakistan, and mostly inbreeding coefficient was high among families.

Conflicts of Interest

The authors have declared that no competing interests exist.

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