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Fluorescence in Situ Hybridization (FISH) in Prader-Willi Syndrome

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ABSTRACT

Introduction: Prader-Willi syndrome (PWS) present in 1/15,000–1/30,000 individuals that is caused by the loss of paternally expressed genes on chromosome 15q11.2-13. Fluorescence in situ hybridization (FISH) is first line of investigation whenever there is strong suspicion of PWS. Diagnosis of Prader Willi Syndrome is very important in multidisciplinary management of patients.
Aims and objectives: To study application of FISH in diagnosis of Prader Willi Syndrome.
Materials and Methods: Peripheral blood samples of 15 patients referred to Division of Human Genetics, Department of Anatomy with suspected Prader Willi Syndrome was subjected to FISH.
Results: Out of 15 samples, three patients were positive for Prader Willi Syndrome. Hence in 20% cases FISH showed a positive result.
Conclusion: FISH is a first line investigation for detection of PWS since it is a rapid and reliable procedure. However negative results should be subjected to molecular genetic testing to confirm diagnosis.

Keywords: FISH, microdeletion, Prader Willi Syndrome, SNRPN, obesity

INTRODUCTION

Microdeletion syndrome is a syndrome caused by chromosomal deletion smaller than 5 million base pairs (5 Mb) span several genes that is too small to be detected by conventional cytogenetic methods or high resolution karyotyping (2–5 Mb). One of common microdeletion syndrome is Prader-Willi syndrome (PWS) present in 1/15,000-1/30,000 individuals that is caused by the loss of paternally expressed genes on chromosome 15q11.2-13.[1] In newborns, symptoms include hypotonia, poor feeding, and slow development. Beginning in childhood, the child will present with hyperphagia, obesity, hypogonadism, hyperactivity and type 2 diabetes. Fluorescence in situ hybridization (FISH) is a gold standard investigation whenever there is strong suspicion of PWS. A number of genes have been mapped within this region, the critical one for PWS is SNURF/SNRPN gene, which contains 10 exons, many snoRNAs, the imprinting centre (IC) and the open reading frame (ORF) of the gene. Of these, exon 1 is critical for imprinting and SNORD 116 RNA is important for many of the clinical features of PWS. PWS is maternally imprinted and is caused by loss of paternal alleles within this region. The genetic mechanisms leading to PWS are by 3 ways - either there is a deletion of the critical region (~75% of patients), uniparental disomy (UPD) (~25%) or an imprinting centre (IC) defect (~1% of PWS).[2] Diagnosis of Prader Willi Syndrome is very important in multidisciplinary management of patients.

Aims and objectives:
To study application of FISH in diagnosis of Prader willi Syndrome.

MATERIALS AND METHODS

Peripheral blood samples of 15 patients referred to Division of Human Genetics, Department of Anatomy for FISH with suspected Prader Willi Syndrome was subjected to standardized lymphocyte culture followed by harvesting and slide

preparation. Prader-Willi/Angelman (SNRPN) localized to 15q11.2 in red and 15q26.3 (sub-telomeric) as control probe for 15 in green from Cytocell Aquarius UK was applied. Analysis was done using fluorescent microscope.

RESULTS

Out of 15 samples, three patients were positive for Prader Willi Syndrome.

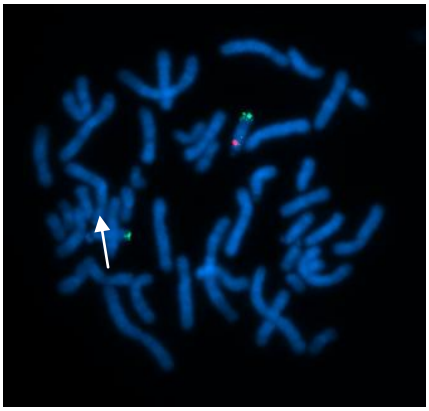

Figure 1: Metaphase spread with one red signal indicating deletion on SNRPN15q11-13 in a patient suspected with Prader Willi Syndrome.

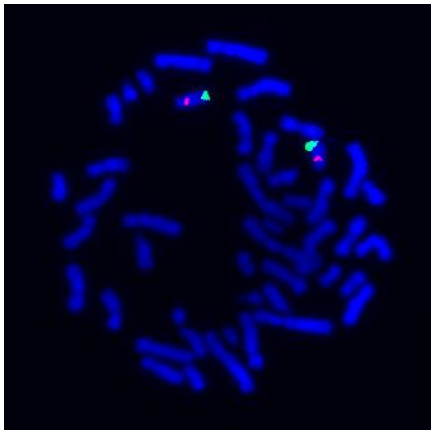

Figure 2: Metaphase spread showing two green signals on 15qter and two red signals on SNRPN15q11-13 in a patient suspected with Prader Willi Syndrome

DISCUSSION

In present study about 20% of cases were positive for PWS. Maria Puiu et al. did a preliminary study in Romania on 9 patients with suspected PWS. They did clinical scoring, FISH and methylation studies. Out of 9 patients 4 were positive for PWS by FISH and six were positive for DNA methylation studies.[3] They concluded that early recognition and diagnosis is essential in PWS, as complex treatment applied in due time leads to prevention of obesity installation and other redoubtable complications.

In study done by Pangkanon S,[4] out of eighteen PWS patients FISH using DNA probes for loci SNRPN FISH confirmed deletion of chromosome 15q11-q13 in fourteen cases (77%). Four cases (23%) were confirmed to have PWS resulting from maternal uniparental disomy by demonstrating exclusively maternal specific DNA methylation patterns. This shows that precise diagnosis will yield a better result with FISH results.

In an Indian study done by Pankaj et al., out of 53 pateints with suspected PWS 8(15%) had microdeletion detected by FISH analysis.[1] In another Indian study done by Haldar et al., at AIIMS, New Delhi, out of 38 cases of suspected PWS only four(10%) were positive for microdeletion by FISH method. Our study correlates with Indian studies stating that clinical accuracy is very important in diagnosis of microdeletion syndromes.[5,6]

In a Thai study done by Wiriyaukaradecha S et al 2 out of 9 patients showed a deletion at 15q11-q13 region by standard GTG chromosome analysis while 4 out of 9(44.4%) patients showed a deletion in this region by FISH. They suggested that methylation specific PCR is suggested to confirm PWS when patient fulfills clinical criteria.[7]

Negative results in FISH for Prader Willi Syndrome will not rule out diagnosis. In cases of strong clinical suspicion molecular genetic testing needs to be done to confirm diagnosis. Molecular genetic testing for Prader-Willi syndrome can be divided into four categories: methylation analysis by Southern blot or polymerase chain reaction (PCR), reverse transcription PCR (RT-PCR) to detect SNRPN expression, microsatellite analysis to detect

uniparental disomy and specialized studies using various molecular techniques for the identification of imprinting defects. However differential diagnosis of Bardet Biedal, Alstrom, Fragile X and Cohen Syndrome should be ruled out.

CONCLUSION

FISH is a first line investigation for detection of PWS since it is a rapid and reliable procedure. However negative results should be subjected to molecular genetic testing to confirm diagnosis.

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