Original Article

Received on 16.07.2016, **Accepted on** 03.08.2016

Indian Journal of Anatomy
Volume 5 Number 3, September - December 2016
DOI: http://dx.doi.org/10.21088/ija.2320.0022.5316.23

Molecular Genetic study of Complete Hydatidiform Moles

J.E. Waghmare*, P.S. Ambulkar, N.M. Gangane***, A.K. Pal******

Abstract

Moles are abnormal pregnancies posing a reproductive health risk and are of two types, Complete hydatidiform moles (CHM) and partial hydatidiform moles (PHM) also commonly known as 'gestational trophoblastic disease'. The genetic constitution of complete hydatidiform mole is either 46,XX or 46,YY (uniparental paternal isodisomy) and 46,XY (uniparental paternal heterodisomy). Till date it is considered that CHM with 46,YY are rarely reported and such with 46,YY karyotype are more neoplastic and less viable, but extensive work is needed in developing and underdeveloped countries. PHMs are generally triploid having maternal contribution also, the genetic constitution of PHM is generally 69,XXY, 69,XXX or 69,XYY. The molar pregnancies are malignant in nature and its neoplasticity is more virulent in CHM as compared to PHM. Difference in the neoplasticity of CHM and PHM is due to its genetic constitution. There are reports where ultrasonic and pathologic investigations have failed to correctly diagnose molar pregnancies in first trimester. Even, sometimes β - hCG levels, which are hallmark for diagnosis shows false negativity. Hence, for confirmation and treatment of such high risk disease genetic diagnosis is must. We conducted molecular genetic study by polymerase chain reaction (PCR) on the genetic constituents of 10 complete moles to confirm their genetic constitutions.

Keywords: Complete Hydatidiform Mole; Patial Hydatidiform Mole; Neoplasticity; Genetic Constitution.

Introduction

Hydatidiform moles, are abnormal pregnancies, develop from an abnormal growth of trophoblastic cells that would otherwise normally develop into placenta. The name hydatidiform mole comes from the greek word 'hydatisia' which means drop of water and "mola" means false conception. They are of two types, Complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM) and both are commonly known as 'gestational trophoblastic disease [1].

Complete hydatidiform moles are resulted from two haploid sets of paternal genomes with no maternal genomic contribution and foetal development or foetal tissues [2,3]. It often present with vaginal bleeding, ultrasound examination shows widespread and marked hyperplasia with swollen villi. It can be distinguished from partial moles by morphology, genetics(Partial moles are triploid) and immune staining of maternally expressed genes [1,4,5]. CHM most often have the karyotype 46,XX or 46,YY with both haploid sets chromosomes being paternally derived [2]. PHMs are generally triploid having maternal contribution also, the genetic constitution of PHM is generally 69XXY, 69XXX or 69XYY [2,6].

Mostly CHM prengnancies cases are sporadic, only a less number of cases are recurrent and often familial, generally diploid and biparental (contain paternal and maternal sets of chromosome). Two genes such as NLRP7 (NLR family, pyrin domain containg 7) and KHDC1 (KH domain containg 3-like) are identified as recessive gene responsible for recurrent hydatidiform moles [3]. Chromosomal and genetic anomalies have a significant adverse effect on human reproduction resulting in infertility, pregnancy loss, still births and molar pregnancy.

Author's Affiliation: *Associate Professor, **Senior Research Fellow, Cytogenetic Division, ****Professor, Cytogenetics and Molecular Genetics, Department of Anatomy, ***Professor and Head, Department of Pathology, MGIMS, Sevagram.

Corresponding Author: A.K. Pal, Cytogenetics and Molecular Genetics, Dept. of Anatomy, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha- 442102.
E-mail: jewaghmare@mgims.ac.in, asokepal@yahoo.com

Genetic investigations can provide important information on reproductive health risk, one such risk is molar pregnancy. A complete hydatidiform mole is a rare finding, however it can develop into gestational tropoblastic neoplasia, a malignant and life threatening disease. We have conducted a molecular genetic study of complete hydatidiform moles to see the frequency of different genetic constituents i.e. 46,XX or 46,YY.

Materials & Method

As it is a rare disease (0.5 to 1/1000 pregnancies) we could collect 10 samples of CHM from our pathology department, further it was used for DNA extraction by polymerase chain reaction (PCR).

Tissue Collections and Processing

Paraffin block were trimmed to remove extra paraffin and placed in xylene overnight. Give repeat changes of xylene for 2 hours to remove any traces of wax. Give three times change of absolute alcohol to remove xylene from the tissue and kept in 90% alcohol for overnight. Next day keep in 70% alcohol for 2 hrs and wash with tap water and kept immersed for 2 hrs by changing the water twice during this period. Wash tissue with PBS buffer for removing impurities.

DNA Extraction from Tissue

Total DNA from mole tissue was extracted according to following standard protocol. Take tissue in micro-centrifuge tube and minced it, add TE9 buffer in tube, kept for 37^{0}C for 24 hrs then remove supernatant and add 350 µl TE9 and add 50 µl of 20% SDS, 0.5% triton X 100, 15 µl of DTT and 25 µl of proteinase K and incubate at 55^{0}C in a water-bath for 24 hrs. After digestion, the lysate was extracted with phenol, followed by phenol/chloroform (1:1v/v) and one-tenth volume of 3M sodium acetate (pH5.6) and incubated at -20^{0}C overnight. After washing with 75% ethanol (v/v), the DNA pellet was dried by speed vacuum, and finally DNA pellet dissolved in 10 mM Tris-Hcl pH 8.3. Now, DNA sample was ready for further analysis.

Identification of Sex Chromosome Genotype by Polymerase Chain Reaction

In polymerase chain reaction study, specific primers were used for amplification of SRY gene on Y chromosome and DAX gene on X chromosome for the detection of X and Y chromosome. SRY gene primer sequences were followed as forward primer: 5'-GAATATTCCCGCTCTCCGGA-3', reveres primer: 5'-GCTGGTGCTCCATTCTTGAG-3' and DAX-1 gene primer sequences were followed as forward primer: 5'-CCGCGCCCTTGCCCAGACC-3, reveres primer: 5-GCCGCCTGCGCTTGATTTGT-3'. Segment of gene was amplified from 100 ng of mole tissue DNA in a 50 µl reaction mixture containing 200 µM of each dNTP, 1µM each of forwards and reverse primers, 1IU of Taq DNA Polymerase, 50 mM KCL, 2 mM $Mgcl_2$, 25 mM, and 10 mM Tris-HCl, pH 8.3. Each PCR was carried out for 30 cycles in a DNA thermal cycle using the standard protocol. PCR conditions of denaturation at 94^{0}C for 1 min, annealing at 55^{0}C for 1 min, and primer extension at 72^{0}C for 1 min after the required no. of cycles, an additional extension step at 72^{0}C for 3 min was performed. PCR product was checked on 1.5% agarose gel using 1x TAE buffer at 150 volts for 1.5 hrs using ethidium bromide containing agarose gel electrophoresis. After gel electrophoresis, gel documentation system was used for detection of SRY or DAX gene. Amplification of desired gene was detected on the basis of 100 bp DNA ladder (i.e. SRY-472 bp and DAX1-835bp) and positive control of DNA bands. Gel photography was done by gel documentation software.

Results

In our study, we analyzed 10 sample of

Table 1: PCR amplification results and genetic constitution of tissues

No. of Tissues	SRY Amplification	DAX-1 Amplification	Chromosome constitution	Remarks
Tissue-1	+	-	YY	Complete mole
Tissue -2	+	-	YY	Complete mole
Tissue -3	+	-	YY	Complete mole
Tissue -4	+	+	XY	Complete mole
Tissue -5	+	-	YY	Complete mole
Tissue -6	+	-	YY	Complete mole
Tissue -7	+	-	YY	Complete mole
Tissue -8	+	+	XY	Complete mole
Tissue -9	+	-	YY	Complete mole
Tissue -10	+	-	YY	Complete mole

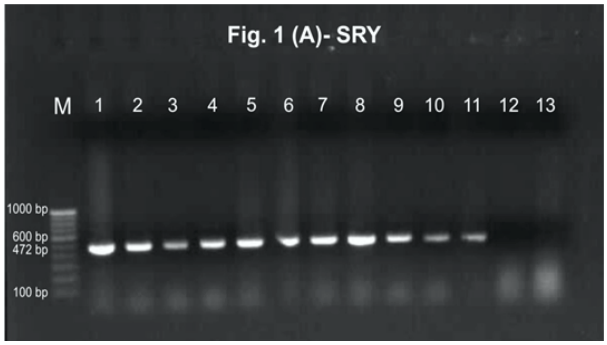

Fig. 1: A: Agarose gel electrophoresis analysis showed the amplification of SRY gene in hydatidiform mole tissue. Lane M-100 bp DNA ladder, Lane No. 1: positive control DNA of fertile male, Lane No.2-11: SRY amplification product from mole tissue DNA, Lane No.12: Negative control water sample, Lane No. 13: Negative control female DNA.

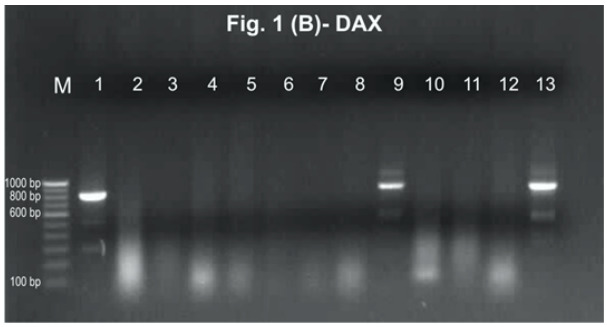

Fig. 1B: Agarose gel electrophoresis analysis showed the amplification of DAX-1 gene in hydatidiform mole tissue. Lane M-100 bp DNA ladder, Lane No. 1: internal positive control DNA of fertile male, Lane No.2-8,10,11: No amplification of DAX-1 product from mole tissue DNA, Lane No. 9: DAX-1 amplification product from tissue DNA, Lane No.12: Negative control water sample, Lane No. 13: positive control female DNA.

hydentidiform mole tissue for identification of XX and YY chromosome genotype by PCR amplification. All 10 mole samples shown complete hydentidiform mole type. Out of 10 samples, 1 CHM tissue shown XY genotype and 9 CHM tissue shown YY genotype (Table 1, Figure 1).

Discussion

Reports on the incidences of complete and partial hydatidiform moles are variable. IN Europe and North America the incidence ranges from 0.6 to 1.1 per 1000 pregnancies, in Australia ranges from 0.91-1.41 per 1000 pregnancies but in Southeast Asia and Japan the incidence is as high as 2.0 per 1000 pregnancies [1,7]. Women younger than 16 have a 6 fold increased risk and women older than 40 have 5 to 10 fold increased risk of mole development compared to women aged 16-40 years [1]. Maternal age appears to be the main risk factor with women at either end of the reproductive age having the highest risk [8,9,10]. Women aged over 50 years have a 1 in 3 risk of complete molar pregnancies. History of previous complete mole is also a risk factor and such women have 10-20 fold higher risk than that of the general population [11].

The abnormal foetal-placental development in a complete mole is due to abnormal effect of genomic imprinting. Loss of the maternal epigenetic imprint and abnormal gains of paternally imprinted gene expression together result in global genome demethylation and abnormal gene expression resulting in abnormal trophoblastic development [3,10]. Two genes NLRP7 and KHDC3L are identified responsible for recurrent hydatidiform moles which is a rare condition. NLRP7 is located at 19q13.4 and it was the first identified recessive gene involved in recurrent hydatidiform moles and the second is KHDC3L, located at 6q13 [2,3]. The exact causal mechanisms involving these two genes are not fully understood. However, possibly deregulation of imprinted genes result in aberrant cell proliferation and differentiation leading to pathogenesis [3]. Accurate diagnosis of a complete mole is of clinical importance because of the increased risk (18-29%) of a developing gestational trophoblastic neoplasia.

In present study, we have studied the frequency of genetic constituents Complete hydatidiform mole. We studied the 10 CHM samples for PCR based screening of sex gene identification by DNA extraction. Here, we observed 9 cases of YY genotype and 1 cases of XY genotype. The molar pregnancies are malignant in nature and its neoplasticity is more virulent in CHM as compared to PHM. Difference in the neoplasticity of CHM and PHM is due to its genetic constitution. In our finding we have got karyotype 46,YY predominantly whereas most of the literature from European countries shows commomon karyotype as 46,XX [12]. This might be due to geographical and genetic variation . Due to paucity of molecular genetic labs as well as the specimens of molar pregnancy, there is no genetic data available across India. Elaborative study is needed with larger sample size to standardize our own data for better patient management.

We believe that the management of HM requires an accurate diagnosis that should be based on a histopathology and conclusively supported by a genetic analysis. Even though this diagnostic pathway may put financial burden on patients, we consider that the efforts justify the benefit for the patient's management. Most of the PHM have been misdiagnosed on ultrasound as well as histopathological investigation as incomplete

abortion hence routinely genetic examination should be done in addition to pathological examination [6,9]. Most of the clinicians rely on β -hCG assay pregnancy test for diagnosing hydatidiform mole. Interestingly, chromatographic immunoassays such as qualitative β-hCG assays, may produce false-negative results in the presence of excessively high antigen concentrations in a phenomenon known as the *high-dose hook effect* [13]. Ultimately, it should prevent the development of choriocarcinoma, as most of these patients can be successfully treated with current chemotherapy. There are still few of the patients who dies from this disease or receive inadequate treatment, usually because of a delayed or erroneous diagnosis [8,9,13]. The authenticity of the diagnosis is key for proper counselling and to categorise patient into *short-term* or *longterm* follow-up to minimize the period during which patients are recommended to use contraceptive methods. Accurate line of treatment is of immense importance for all the patients, still it carries greater importance in higher age group. Patients with PHM can conceive after six months but patients with CHM cannot conceive for almost two years, which is traumatic for higher age group [4,14]. The risk of hydatidiform mole increases with higher age and previous history of molar pregnancy [8,11]. As the virulence of the disease changes with its genetic constitution it is of utmost importance to confirm it for treatment and subsequent rehabilitation of the patients.

References

1. Savage P. Clinical features of molar pregnancies and gestational trophoblastic neoplasia. Gestation Trophobl Dis. 2011; 216–48.
2. Bestor TH, Bourc'his D. Genetics and epigenetics of hydatidiform moles. Nat Genet. 2006 Mar; 38(3): 274–6.
3. Nguyen NMP, Slim R. Genetics and epigenetics of recurrent hydatidiform moles: basic science and genetic counselling. Curr Obstet Gynecol Rep. 2014; 3(1): 55–64.
4. Monchek R, Wiedaseck S. Gestational Trophoblastic Disease: An Overview. J Midwifery Womens Health. 2012 May; 57(3): 255–9.
5. Sebire NJ, Seckl MJ. Gestational trophoblastic disease: current management of hydatidiform mole. BMJ. 2008 Aug 15; 337: a1193.
6. Petignat P. Is genetic analysis useful in the routine management of hydatidiform mole? Hum Reprod. 2003 Feb 1; 18(2): 243–9.
7. Bracken MB. Incidence and aetiology of hydatidiform mole: an epidemiological review. BJOG Int J Obstet Gynaecol. 1987 Dec 1; 94(12): 1123–35.
8. Tse Ky , Chan Kk , Tam Kf , Ngan Hy . 20-year experience of managing profuse bleeding in gestational trophoblastic disease. J Reprod Med. 2007 May; 52(5): 397–401.
9. Cavaliere A, Ermito S, Dinatale A, Pedata R. Management of molar pregnancy. J Prenat Med. 2009; 3(1): 15.
10. Sebire N j., Fisher R a., Foskett M, Rees H, Seckl M j., Newlands E s. Risk of recurrent hydatidiform mole and subsequent pregnancy outcome following complete or partial hydatidiform molar pregnancy. BJOG Int J Obstet Gynaecol. 2003 Jan 1; 110(1): 22–6.
11. Banet N, DeScipio C, Murphy KM, Beierl K, Adams E, Vang R, et al. Characteristics of hydatidiform moles: analysis of a prospective series with p57 immunohistochemistry and molecular genotyping. Mod Pathol. 2014; 27(2): 238–54.
12. Lawler SD, Fisher RA, Dent J. A prospective genetic study of complete and partial hydatidiform moles. Am J Obstet Gynecol. 1991 May; 164(5): 1270–7.
13. Hunter CL, Ladde J. Molar pregnancy with false negative â-hCG urine in the emergency department. West J Emerg Med . 2011 ;12(2).
14. Bagshawe KD, Lawler SD. Unmasking moles. BJOG Int J Obstet Gynaecol. 1982 Apr 1; 89(4): 255–7.