Recurrent hydatidiform mole with NLRP7 mutation: The first confirmed case in the Philippines*

By Martin Antonio B. Medina, MD and Agnes L. Soriano-Estrella, MD, MHPEd, FPOGS, FPSSTD

Department of Obstetrics and Gynecology, Philippine General Hospital, University of the Philippines-Manila

ABSTRACT

High gravidity hydatidiform mole (HM) without normal pregnancy is very rare. The challeng of managing such cases will dwell on the concern of having normal conception versus having another molar gestation and its neoplastic sequelae.

Presented in this paper is a case of a 32-year-old, gravida 5 para 0 (0040) who was admitted for the management of her fifth molar pregnancy. She underwent suction curettage and administration of methotrexate chemoprophylaxis. Genetic testing was done, which revealed a homozygous mutation in NLRP7, the gene implicated in recurrent molar gestations. This paper discusses the proper approach to determine the cause of recurrent molar pregnancies, as well as the management and prognosis of such cases.

Keywords: familial hydatidiform mole, recurrenthydatidiformmole, NLRP7 mutation.

INTRODUCTION

Hydatidiform mole (HM), the most common gestational trophoblastic disease (GTD), is a non-viable genetically abnormal conception with excess expression of paternal genes and abnormal proliferation of the placental trophoblast.[1] There are two types of HM, complete and partial which can be differentiated based on morphologic, cytogenetic, and clinicopathologic features. HMs can also be categorized based on the number of molar pregnancies a patient has had. One molar pregnancy is termed as sporadic hydatidiform mole while two or more molar pregnancies is termed recurrent hydatidiform mole (RHM).

High gravidity recurrent molar pregnancy is extremely rare, the highest number of molar gestation in a single patient is 18 and was reported in 1912.[1] This paper discusses the case of a 32-year-old multigravid who presented with her fifth molar pregnancy. Proper approach to determine the cause of her repeated molar gestations, as well as the management and prognosis of such case will be discussed in this paper.

THE CASE

This is the case of a 32-year-old, gravida 5 para 0 (0040) from Majayjay, Laguna who was referred by a private obstetrician-gynecologist for the management of her RHM.

The patient's past medical, family medical, personal-social, and menstrual histories were unremarkable. There were no cases of hydatidiform moles noted in her family from the first up to the third generation (Figure 1).

This is her fifth pregnancy. All her previous pregnancies were hydatidiform moles for which she underwent suction curettage. The age of gestation based on her last menstrual period is 13 weeks.

The patient's history started three weeks prior to admission, when, after experiencing missed menses, pregnancy test was performed which showed a negative result. No consultation was done.

Six days prior to admission, patient had vaginal spotting. She consulted at a local hospital where a transvaginal ultrasound was done, which revealed molar pregnancy. Patient was then advised to consult a trophoblastic disease specialist for further work-up and management, hence subsequent admission at our institution.

At the admitting section, the patient was awake,

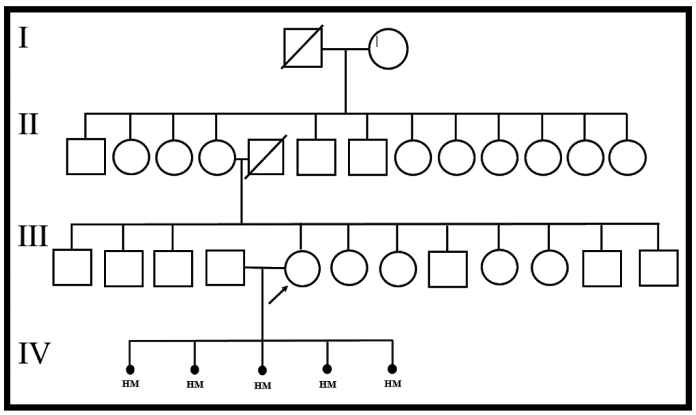


Figure 1. Index patient's family pedigree.

*Finalist, 2017 Philippine Obstetrical and Gynecological Society (POGS) Interesting Case Paper Contest, April 6, 2017, Citystate Asturias Hotel, Puerto Princesa City, Palawan

coherent, ambulatory, and not in cardio-respiratory distress. She had normal vital signs with blood pressure of 100/60 mmHg, heart rate of 84 bpm, respiratory rate of 20 cpm and temperature of 36.8°C. The abdomen is globular and non-tender.

The pelvic examination revealed a normal external genitalia and nulliparous vagina. The cervix was 3 x 2 cm smooth, soft and closed. The corpus was doughy and symmetrically enlarged to about 16 to 18-week size. No adnexal masses nor tenderness noted. Rectovaginal examination was unremarkable

The pregnancy test was positive. The baseline laboratory examinations included: complete blood count (CBC), blood typing, serum urea nitrogen, creatinine, electrolytes, aspartate aminotransferase (AST), alanine amino transaminase (ALT), diluted serum ß human chorionic gonadotropin (ß-HCG), thyroid function test, chest radiograph, and transvaginal ultrasound (Table 1). Results showed an elevated diluted serum ß-HCG of 551, 100.00 mIU/mL. She also had an elevated AST and ALT which were 16 times and 14 times elevated than the normal, respectively. Urinalysis revealed bacteriuria. Her serum thyroid stimulating hormone (TSH) was low with high normal free thyroxine (FT4). The transvaginal ultrasound (TVS) revealed that the uterus was anteverted with globular contour and homogenous echopattern measuring 16.8 x 11.6 x 9 cm. The cervix measured 3.1 x 2.6 x 3.32 cm with homogenous stroma and distinct endocervical canal. The endometrial cavity was dilated by a heterogeneous mass measuring 10.7 x 10.8 x 6.9 cm (volume of 418cc), with multiple sonoluscent cystic spaces within. The myometrium was thinnest at the posterior fundal area measuring 1.1 cm. No myometrial invasion was noted. The subendometrial halo was intact. The right ovary was not visualized. The left ovary measured 3.1 x 3.9 x 0.9cm. There were no adnexal masses seen. There was no free fluid in the cul-de-sac. The sonologic impression was endometrial mass, consider gestational trophoblastic disease, probably hydatidiform mole; normal left ovary (Figure 2).

The initial working impression was recurrent hydatidiform mole, asymptomatic bacteriuria. The patient was then admitted for suction curettage and chemoprophylaxis. On suction curettage, about 400cc of vesicular materials with no fetal tissues nor normal appearing-placental tissue were evacuated (Figure 3A). The specimen was sent for histopathologic studies. Chemoprophylaxis in the form of Methotrexate 0.3mg/kg body weight was given once daily for five days. Antibiotic coverage for asymptomatic bacteriuria was also given in the form of Cefuroxime 500mg/tablet every 12 hours for seven days. Repeat diluted serum ß-HCG obtained a week after molar evacuation revealed a value of 2,109.10 mIU/

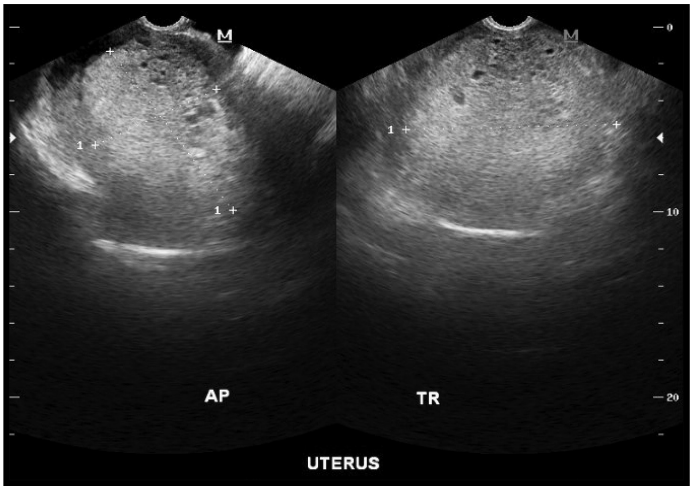


Figure 2. Transvaginal ultrasound of the patient revealing a heterogenous mass with multiple sonuluscent cystic spaces measuring 10.7 x 10.8 x 6.9 cm (volume of 418cc) occupying the endometrial cavity.

mL. The patient was advised serial ß-HCG determination and contraception during the period of monitoring. The histopathologic result of the suction curettage specimen revealed complete hydatidiform mole and the sharp curettage specimen showed decidual tissues (Figure 3B).

In order to detect a possible genetic cause for the RHM, blood samples from the patient, her husband, mother, grandmother and sister were sent to a genetics laboratory in Canada. Results revealed that the patient has an NLRP7 mutation by genomic DNA amplification of the 11 exons and sequencing in the two directions. This analysis revealed the presence of a previously reported mutation, c.2571_2572dupC, predicted to lead to a protein truncation, p.Iso858Hisfs*11, in a homozygous state. Moreover, her sister was also found to have homozygous mutation of NLRP7 gene and her mother was a carrier of the mutation in a heterozygous state (Figure 4).

The results were thoroughly explained to the patient and her family. Her risk for another RHM and possible neoplastic sequelae were emphasized. Latest ß-HCG titer

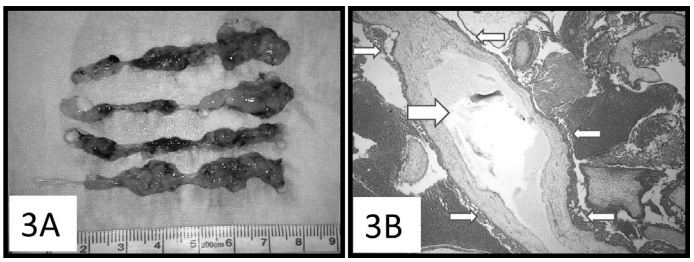
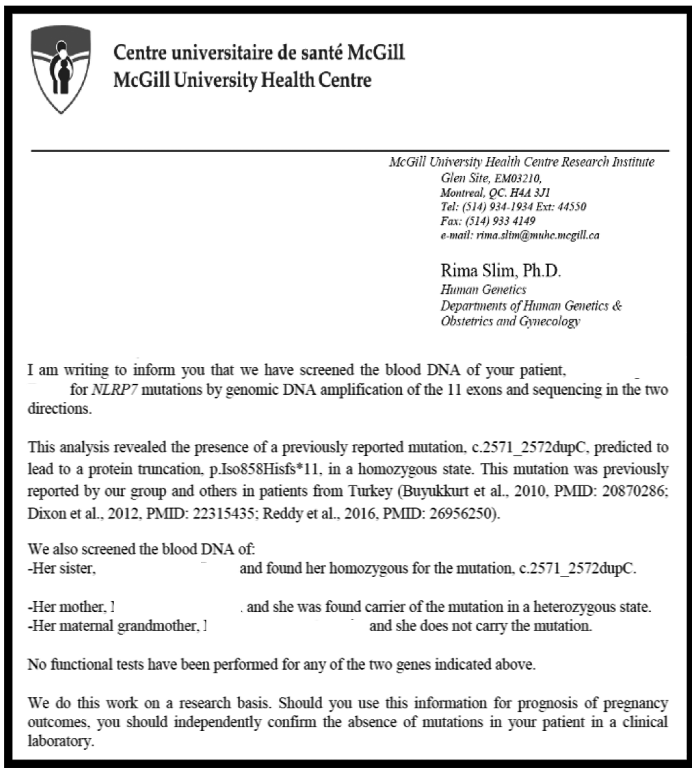


Figure 3. Gross and microscopic pictures of the specimen. (3A) The suction curettage specimen showing multiple vesicular materials admixed with blood clots. (3B) Low power magnification of the specimen showing hydropic villi (yellow arrow) with peripheral proliferation of trophoblast (white arrows).

of the patient is 1.20mIU/mL and she remains compliant with her follow-up consultations and ß-HCG monitoring.



Centre universitaire de santé McGill
McGill University Health Centre

McGill University Health Centre Research Institute
Glen Site, EM03210,
Montreal, QC. H4A 3J1
Tel: (514) 934-1934 Ext: 44550
Fax: (514) 933 4149
e-mail: rima.slim@muhc.mcgill.ca

Rima Slim, Ph.D.
Human Genetics
Departments of Human Genetics &
Obstetrics and Gynecology

I am writing to inform you that we have screened the blood DNA of your patient, for *NLRP7* mutations by genomic DNA amplification of the 11 exons and sequencing in the two directions.

This analysis revealed the presence of a previously reported mutation, c.2571_2572dupC, predicted to lead to a protein truncation, p.Iso858Hisfs*11, in a homozygous state. This mutation was previously reported by our group and others in patients from Turkey (Buyukkurt et al., 2010, PMID: 20870286; Dixon et al., 2012, PMID: 22315435; Reddy et al., 2016, PMID: 26956250).

We also screened the blood DNA of:
-Her sister, and found her homozygous for the mutation, c.2571_2572dupC.

-Her mother, , and she was found carrier of the mutation in a heterozygous state.
-Her maternal grandmother, and she does not carry the mutation.

No functional tests have been performed for any of the two genes indicated above.

We do this work on a research basis. Should you use this information for prognosis of pregnancy outcomes, you should independently confirm the absence of mutations in your patient in a clinical laboratory.

Figure 4. NLRP7 genetic test result

DISCUSSION

Hydatidiform mole is the most common form of gestational trophoblastic disease occurring in about 1 in 500–1000 pregnancies. The incidence varies widely, depending on the geographic location. Estimates from studies conducted in North America, Australia, and Europe have shown the incidence of hydatidiform mole to range from 0.57–1.1 per 1000 pregnancies, whereas studies in Southeast Asia and Japan have suggested an incidence of 2.0 per 1000 pregnancies.[1]

Studies have shown that after a molar pregnancy, the risk of another molar pregnancy rises to 1-2%.[2] After two molar gestations, the risk for a third mole is 15-20%.[1] Review of literatures also showed that the highest frequency of molar pregnancy in a single patient is 18 as reported by Essen-Moeller in 1912.[1] More recently, patients with seven and six consecutive molar pregnancies were reported in the years 2001 and 2011 respectively.[3,4] In our institution, based on review of records, the earliest documented case of RHM was in 1983 with the patient having two molar pregnancies complicated with choriocarcinoma. Although similar cases have been sporadically documented in the succeeding years, the first case study published regarding high gravidity molar pregnancy was reported by Cristi et al. in 2006.[5] To date, the highest frequency of molar pregnancy recorded in our institution is of our index patient who had five consecutive molar gestations without a normal pregnancy.

Central to the diagnosis and management of HM is thorough history taking, comprehensive physical examination and appropriate laboratory tests. RHMs have no distinguishing clinical characteristics from the non-recurrent sporadic moles except for the history of repeated molar pregnancies. The goal of management of such cases is to determine the cause of recurrence, ascertain the patient's chance for a normal pregnancy and prevent neoplastic sequelae.

Genotype of HMs are usually androgenic, however, the degree of paternal contribution may vary. In minority of cases, some HMs have been reported to have maternal chromosomes hence are biparental. Diploid biparental genotype are associated with RHMs and often run in families. Familial biparental hydatidiform mole (FBHM) is the only known pure maternal-effect recessive inherited disorder in humans. Affected women, although developmentally normal themselves, suffer repeated pregnancy loss because of the development of the conceptus into a CHM in which extraembryonic trophoblastic tissue develops but the embryo itself suffers early demise. This developmental phenotype results from a genome-wide failure to correctly specify or maintain a maternal epigenotype at imprinted loci.[1] Moreover, these patients appear to have an autosomal recessive condition, causing them to have recurrent molar gestations with very little chance of a successful normal pregnancy.

Most cases of FBHM result from mutations of NLRP7. Wang et al. analyzed the NLRP7 gene in affected individuals from 20 families with a confirmed diagnosis of FBHM and identified 16 different mutations in 17 of the families.[6] This was subsequently confirmed by Fallahian et al. in their genetic analysis of tissues from the CHM patients, which was previously studied by Wang. Patients were found to be homozygous for a 14-bp duplication in the NLRP7 gene. In their study, they concluded that these findings were consistent with a role for NLRP7 in setting and/or maintaining the maternal imprint.[7]

Currently, the two genes that have been identified as the causative agents for the development of RHM are NLRP7 and KHDC3L. NLRP7 is a nucleotide oligomerization domain (NOD)-like receptor, pyrin containing 7, maps to 19q13.4.[1] The family of genes where NLRP7 belongs are commonly expressed in the germline and early embryos, suggesting their involvement in early developmental process.[1] In the study done by Slim and Wallace (2013), they concluded that oocytes from patients with the gene mutation are defective at several levels and are unable to sustain early embryonic development. As a result, the retention of these non-viable pregnancies with no

embryos to later gestational stages leads to the hydropic degeneration of the chorionic villi.[8] Moreover, the gene has also been postulated to have a role in inflammation and apoptosis mainly by down regulation of intracellular IL-1β. IL-1β is responsible for cytokine secretion, hence, its downregulation fail to mount an appropriate inflammatory response to reject the hydropic non-viable gestation as normal women would. Interestingly, a recent study has identified that NLRP7 knockdown accelerates trophoblast differentiation and increase the level of ß-HCG.[1] Although several studies are still being conducted to strengthen the role of NLRP7 mutation, all of these observations lead to the fundamental aspects of recurrent moles.

The second recessive gene responsible for RHMs is KHDC3L (KH domain containing 3-like), which was identified in 2011 by Parry et al.[1,9] KHDC3L maps to chromosome 6 and this accounts for 10–14% of patients with RHMs.[1] KHDC3L transcripts have been identified in several human tissues, including all oocyte stages, preimplantation embryos, and hematopoietic cells. KHDC3L codes for a small protein of 217 amino acids belonging to the KHDC1 (KH homology domain containing 1) protein family, members of which contain an atypical KH domain that does not bind RNA which is an important step in protein synthesis. In humans, expression of KHDC3L is highest in oocytes at the germinal vesicle stage and then decreases during preimplantation development and becomes undetectable at the blastocyst stage similar to the expression prolife of NLRP7.[9] Furthermore, KHDC3L co-localizes with NLRP7 to the microtubule organizing center and the Golgi apparatus in lymphoblastoid cell lines which suggests that the two genes may have similar or overlapping functions in oocyte and early embryonic development.[1]

In the index case, the long term management post a major clinical dilemma. The patient's age, obstetric history and her desire of a normal pregnancy are the foremost considerations in our management.

After surgical evacuation of a molar pregnancy ß-HCG titers eventually fall, however, in 20% of cases it will persistently elevate.[8] An elevated ß-HCG on the background of a mass in the uterus or somewhere else is highly suspicious of a GTN. Most of GTNs are seen after a HM in 60% of cases.[1,8] Acosta-Sison et al. in 1959 concluded in their study that there is an increasing degree of invasiveness with subsequent GTD episodes.[9] Moreover, there is also a tendency towards worsening histology hence an increased incidence of invasive mole and choriocarcinoma in the subsequent trophoblastic episodes.[1] Considering these findings, the index patient was given Methotrexate chemoprophylaxis and advised strict ß-HCG monitoring.

Considering that the cause of molar recurrence is mostly genetic in nature and the remaining is idiopathic, genetic testing plays a vital role in patient management. Phuong Nguyen and Slim proposed an algorithm that can be used in patients diagnosed with RHM (Figure 5). In their work, they have emphasized that the goal of patients seeking DNA testing is to ascertain their chances of carrying normal pregnancies and their risk for mole recurrence and malignant degeneration. They proposed that patients with at least two HMs should be offered DNA testing first for NLRP7.[1] Although genetic testing for patients with history of RHM is the current recommendation, the cost of running the tests and its availability are its main limiting factors. Fortunately, a free genetic test was performed in the index case. Result revealed that the patient as well as her sister have homozygous mutation in NLRP7 gene. Since 2006, there are 69 cases with RHM phenotypes documented having mutations in NLRP7 worldwide (http://fmf.igh.cnrs.fr/ISSAID/infevers/). In the Philippines, this is the first case documented with the genetic mutation.

Patients positive to the DNA test must undergo genetic counseling, a process of communicating information about genetic risks which allows the patient to have an informed decision. In the index case, focus was placed on her susceptibility to having another molar pregnancy, her capability of having normal pregnancy, risk for GTN, and the possible solutions to these risks. With proper and thorough explanation, the patient and her husband agreed to avoid pregnancy by using contraceptive

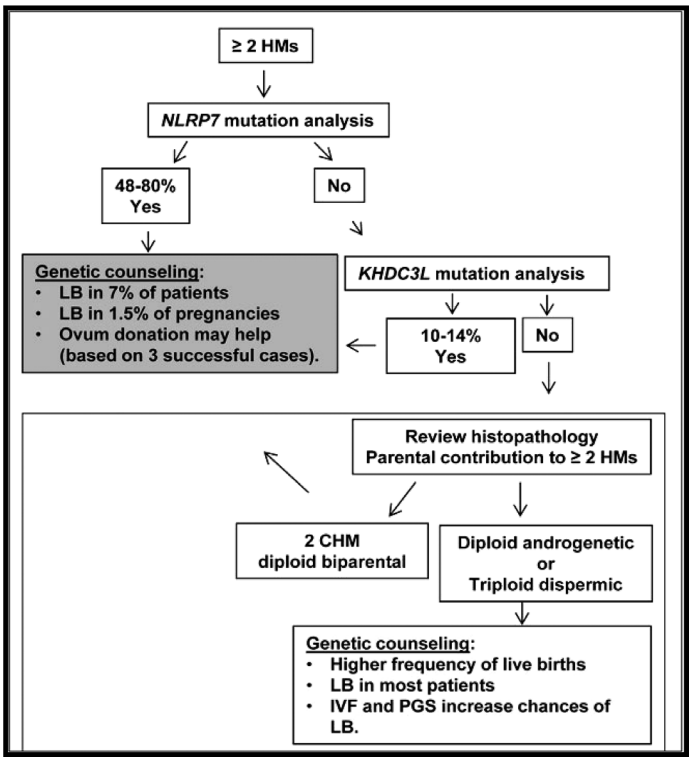


Figure 5. Screening recommendation for genetic testing and counselling of patients with RHM.[1]

pills as a means of family planning. The patient verbalized her understanding and determination for follow-up. The couple's strong family ties serve as their main support group. The patient's sister was also informed that she has a strong risk factor for a RHM.

Only 7% of 43 reported cases of RHM with two defective alleles in NLRP7 had normal spontaneous pregnancy.[1] No other cases of live births have been reported in patients with RHMs harboring NLRP7 or KHDC3L mutations. A patient with two defective alleles in NLRP7 had one livebirth following ovum donation is an exception. Ovum donation may improve patients' reproductive outcomes based on the assumption that the donor of the "normal" ovum has no NLRP7 or KHDC3L mutation. Thus far, few patients with mutations in NLRP7 have tried ovum donation, and three had normal live births, which provides some hope for patients despite the elevated cost of such procedure, its limited accessibility and ethical issues.[1] Reubinoff and colleagues reported a promising approach for the prevention of repeated CHM using intracytoplasmic sperm injection (ICSI) and preimplantation genetic diagnosis (PGD) with fluorescence in-situ hybridization (FISH), unfortunately, pregnancy was not achieved using this proposed method.[11] Patients with the genetic mutation who decide not to be pregnant are given with long term contraceptives or offered with permanent contraception. In the Philippines, where ovum donation is not legal, adoption may be offered.

CONCLUSION

Genetic mutation in NLRP7 has been documented as a cause of recurrent molar gestation in the index case. It is therefore prudent that a general obstetrician-gynecologist with the assistance of a trophoblastic disease specialist emphasize the importance of genetic testingin patients with RHM. A holistic approach is key in managing such cases which includes early medical and surgical intervention, effective family planning method, and religious follow-up. ■

Table 1. Baseline laboratory tests and results.

Hematology/Complete Blood Count		**Blood Chemistry**	
Hemoglobin	120	Blood Urea Nitrogen	6.7
Hematocrit	0.36	Creatinine	79
White Blood Cells	9.17	Blood Urea Nitrogen	6.7
Neutrophil	0.74	Creatinine	79
Platelet	306	Sodium	135
Platelet	306	Potassium	3.1
Coagulation		Chloride	95
		Calcium	2.37
Prothrombin Time (PT)	12.6	Albumin	37
Partial Thromboplastin Time (PTT)	30.6	Aspartate aminotransferase (AST)	584
PT %	111	Alanine amino transaminase (ALT)	752
PT INR	0.94	Lactate dehydrogenease (LDH)	1064
Urinalysis		**Immunology**	
White Blood Cells	4	Free T4	48.57
Bacteria	1179	Thyroid Stimulating Hormone	0.0064
Epithelial Cells	66		

REFERENCES

1. Nguyen N, Slim R. Genetics and Epigenetics of Recurrent Hydatidiform Moles: Basic Science and Genetic Counselling. Curr Obstet Gynecol Rep. 2014; 3(1):55-64.

2. Barroilhet L, Garrett L, Bernstein M, Esselen K, Goldstein D, Berkowitz R. Update on subsequent pregnancy outcomes in patients with molar pregnancy and persistent gestational trophoblastic neoplasia. *Gynecologic Oncology*. 2012; 125:S83-S84.

3. Al-Ghamdi A. Recurrent hydatidiform mole: A case report of six consecutive molar pregnancies complicated by choriocarcinoma, and review of the literature. *J Fam Community Med*. 2011; 18(3):159.

4. Ozalp S, Yalcin O, Tanir H, Etiz E. Recurrent Molar Pregnancy: Report of a Case with Seven Consecutive Hydatidiform Moles. *Gynecologic and Obstetric Investigation*. 2001; 52(3):215-216.

5. Cristi M, Jacinto E, Manalastas R. Recurrent hydatidiform mole: A case report. *Philipp J Obstet Gynecol*. 2006; 30(4):204-210.

6. Wang C, Dixon P, Decordova S, Hodges M, Sebire N, Ozalp S et al. Identification of 13 novel NLRP7 mutations in 20 families with recurrent hydatidiform mole; missense mutations cluster in the leucine-rich region. *Journal of Medical Genetics*. 2009; 46(8):569-575.

7. Fallahian M, Sebire N, Savage P, Seckl M, Fisher R. Mutations in NLRP 7 and KHDC 3 L Confer a Complete Hydatidiform Mole Phenotype on Digynic Triploid Conceptions. *Human Mutation*. 2012; 34(2):301-308.

8. Slim R, Wallace E. NLRP7 and the Genetics of Hydatidiform Moles: Recent Advances and New Challenges. *Front Immunol*. 2013; 4.

9. Acosta-Sison H. The chance of malignancy in a repeated hydatidiform mole. *Am J Obstet Gynecol*. 1959; 78:876-7.

10. Reubinoff B, Lewin A, Verner M, Safran A, Schenker J, Abeliovich D. Intracytoplasmic sperm injection combined with preimplantation genetic diagnosis for the prevention of recurrent gestational trophoblastic disease. *Human Reproduction*. 1997; 12(4):805-808.