Viral Etiology of Complete Hydatidiform Moles (CHM)

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Complete hydatidiform moles (CHM) are the most common forms of gestational trophoblastic disease. CHM prevalence rate is higher in Kerala, India as compared to other parts of the world. The etiology of this disease is not yet clearly understood. Reports and observations suggest conceptual alterations, which could be due to involvement of viruses or carcinogens rather than host factors. In this study, the association of common genital viruses with this disease was examined in 105 cases of CHM and 95 cases of normal placentae using immunohistochemistry, ELISA and PCR techniques. Present study suggests an association of human papilloma virus (HPV) infection with this disease while no association was noticed with other genital viruses such as Human immunodeficiency virus (HIV), Cytomegalovirus (CMV) and Herpes simplex virus (HSV). This is the second report in world literature and first in Indian literature showing such an association.

Keywords: CHM, HIV, HPV, HSV, CMV

Introduction
Complete hydatidiform moles (CHM) are post-conceptual complications of pregnancy affecting women of the reproductive age group. The incidence of CHM shows wide variations in different parts of the world, with Kerala reporting a high hospital prevalence rate of 12/1000 deliveries (Molykutty et al, 1993). The etiology of this disease is not much studied. In CHM, host factors seem to play minimal etiological role as it is the single pregnancy that is affected. The infected individuals, most often, have normal pregnancies before and after the molar pregnancy (Kim et al, 1998). This gives credence to the hypothesis that the conceptus is affected by a virus/carcinogen in the period between trophoblast development and differentiation and the latent survival of such altered cells. However, studies on the association of viruses with this disease are very limited.

Transplacental transmission of viruses, human immunodeficiency virus (HIV), cytomegalovirus (CMV), herpes simplex virus (HSV) and human papilloma virus (HPV), have been reported (Muhlemann & Menegus 1996; Shearer et al, 1997; Halwachs-Baumann et al, 1998; Jun et al, 2000). MEDLARS 1988-2001 (search terms used-complete hydatidiform mole, placenta and virus) has, however, revealed only two reports on association of viruses with hydatidiform moles and choriocarcinoma (Larsson et al, 1994; Pao et al, 1995). In the present study, the involvement of common genital viruses with the pathogenesis of hydatidiform mole was analysed.

Materials and Methods
Viral association was evaluated in 105 cases of CHM and 95 cases of normal placentae using immunohistochemistry, ELISA and PCR techniques.

Sample Collection
Fresh tissue samples and blood were collected from patients undergoing suction evacuation and normal term delivery at the Sree Avittam Thirunal Hospital for Women and Children, Kerala, India. Informed consent was obtained from all the patients of this study. A part of the tissue was snap frozen in liquid nitrogen for PCR analysis, and one part was fixed in 10% buffered formalin for immunohistochemistry. The serum was stored at −20°C for ELISA technique.

Immunohistochemistry
Immunohistochemical expression of the viral antigens, CMV (Novacastra, UK), HSV (Dako, Denmark) and HPV E6 protein (Santa Cruz Biotech, California) were evaluated on 5µ deparaffinised sections using avidin biotin complex peroxidase assay and graded based on the intensity of staining.
independently by two observers (SA and BP) as follows: 1-Negative, 2-Mildly positive, 3-Moderately positive and 4-Intensely positive.

**ELISA**

ELISA was carried out for the detection of HIV (Ranbaxy Diagnostics, New Delhi), CMV (Sigma Diagnostics, USA) and HSV (Rashmi Diagnostics, Bangalore) infection using commercially available ELISA kits.

**PCR**

Frozen samples (approximately 2 mg) were minced and suspended in 400 μl of digestion buffer containing 10 mM Tris, HCl, pH 8.3; 25 mM EDTA; 0.5% SDS and 0.1 mg/ml proteinase K and incubated overnight at 56°C. The lysate was extracted with a phenol:chloroform:isoamyl alcohol mixture (25:24:1), ethanol precipitated and dissolved in 50 μl of sterile distilled water. Of this, 1-2 μl was used for each PCR amplification.

All the DNA samples were amplified with β-globin primers to assess DNA integrity and samples that tested positive were further analysed. PCR reactions were performed in 40 μl volumes containing 10 mM Tris, HCl, pH 8.3; 50 mM KCl; 1.5 mM MgCl₂; 200 μM dNTPs; 2.5 units Taq polymerase (Gibco BRL, USA) and 4 μM primers. Thermocycling was done in a Peltier thermal cycler (PTC-200) and involved and initial denaturation step at 95°C for 3.5 min, followed by 40 cycles at 95°C for 90 sec, 50°C for 90 sec, 72°C for 120 sec, with a final elongation step at 72°C for 10 min. HPV DNA was identified using the consensus primers MY09 and MY11 (conserved region of L1 gene, 450 bp) [MY09 5' GCACAGGGCATATAAATGG, MY11 5' CGTCCAAAGGGGAAACTGATC] from Gibco BRL, USA. Distilled water instead of DNA was used as negative control. DNA from HPV positive genital warts served as positive control. PCR products were analysed on 1% agarose gel and visualized under ultraviolet light on a UV-Visible transilluminator (UV-25, Hoefer Pharmacia Biotech Inc., USA).

**Statistical Analysis**

Staining intensity (score) was expressed as mean score and standard error. The mean staining score was calculated using Student's 't' test. Statistical analysis to compare the staining intensities of the various antibodies in CHM and normal placentae was done using Chi Square test and non-parametric Mann-Whitney U-Wilcoxon Rank Sum test. p value < 0.05 was considered significant.

**Results and Discussion**

Based on published reports and the hypothesis that viral infection could be an etiological factor in CHM, association of viruses was evaluated in CHM and normal placentae. The study on HIV (Zachar et al, 1991) suggests permissiveness of transformed trophoblast cells to HIV. In the present study, only one serum sample of CHM was HIV positive. None of the normal serum samples tested was HIV positive. In western countries, HIV seroprevalence among the child bearing women is generally under 1%, whereas in developing countries, the seroprevalence among pregnant women can be as high as 32% (De-Ruiter & Brocklehurst, 1998). Even though situated in a developing country, Kerala has a low HIV prevalence (Legori et al, 1998). In Thiruvananthapuram, the prevalence rate of HIV is 10/100,000 individuals and in pregnant women this is about 5/100,000 pregnancies. Present study supports the low prevalence rate of AIDS in Kerala and also suggest that HIV infection cannot be considered an etiological factor in CHM (Enose et al, 1998). Pregnancy protective factor (PPF) is a peptide found in the urine of pregnant women in close association with human chorionic gonadotropin, hCG. It is hypothesised that PPF if present in women in whom gestational trophoblastic disease antecedes exposure to HIV should rarely, if ever, also exhibit HIV (Albini et al, 1997; Lunardi-Iskandar et al, 1998). This may also account for the low prevalence rate of HIV in women with CHM.

CMV is the most common cause of congenital viral infection (Nigro et al, 1993; Laifer et al, 1995). It is usually acquired through the oral-respiratory route, sexual contact or by blood transfusion. Infected trophoblast may be involved in maternofetal transmission of human CMV (Halwachs-Baumann et al, 1998). Jun et al (2000) have suggested a molecular mechanism involving human CMV gene products US3 and US6 in the down-regulation of trophoblast class I MHC molecules, which may contribute to fetal loss.

In present study, the percentage of cases showing tissue positivity for CMV antigen using antibody to CMV did not show considerable difference in normal placentae and CHM (Table 1). The positive staining was generally of mild to moderate intensity with no significant difference in the staining intensities between the normal placentae and CHM (Table 1, Fig. 1 A & B).

The ELISA titres for circulating antibodies against CMV paralleled the immunohistochemical finding and did not show much difference in normal placentae and CHM. The mean titre was slightly higher in the normal
placentae (1.40 ± 0.19 in normal placentae vs 1.20 ± 0.09 in CHM). About 15% of normal placentae and 18% of CHM were positive for CMV.

The high levels of interferon in placenta, maternal and cord blood suggest a protective effect against intrauterine HSV infection (Zolravkovic et al., 1997). Intrauterine infection with HSV has been associated with a significant number of neonatal HSV infections and high morbidity rates (Lanouette et al., 1996). Intrauterine co-infections with HSV and CMV have also been reported (Muhlemann & Menegus, 1996). So the authors investigated the association of HSV with CHM.

In this study, the immunohistochemical staining with HSV antibodies did not show any significant difference in the normal placentae and CHM. The positive staining pattern was mild to moderate in both normal placentae and CHM (Fig. 1 C & D). The percentage of cases showing positivity was almost similar in both the groups (Table 1). The difference in the mean staining scores in the normal placentae and CHM was not statistically significant (Table 1).
Fig. 1G — Gel showing PCR products of samples using HPV primers
Lanes 1, 3, & 8 to 11: Amplified DNA of CHM showing intense bands
Lanes 2, 4, & 5: Amplified DNA of normal placentae showing faint bands
Lane 6: Hae III digested pBR 322 DNA molecular weight marker
Lane 7: Negative control
Lane 12: Positive control showing very intense bands (Amplified DNA from genital wart containing HPV).

Table 1 — Immunohistochemical staining using viral antibodies in normal placenta and CHM

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<tr>
<th></th>
<th>CMV</th>
<th>HSV</th>
<th>HPV</th>
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<tr>
<td>% positive cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N. Placenta</td>
<td>11</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>CHM</td>
<td>15</td>
<td>8</td>
<td>39</td>
</tr>
<tr>
<td>Chi square value</td>
<td>NS*</td>
<td>NS</td>
<td>0.0001</td>
</tr>
<tr>
<td>Staining Score</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N. Placenta</td>
<td>4.58 ± 0.67</td>
<td>3.96 ± 0.54</td>
<td>3.32 ± 0.45</td>
</tr>
<tr>
<td>CHM</td>
<td>4.86 ± 0.78</td>
<td>4.45 ± 0.82</td>
<td>5.95 ± 0.54</td>
</tr>
<tr>
<td>p value</td>
<td>NS</td>
<td>NS</td>
<td>0.03</td>
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*NS-Not significant; Mean ± SE

Observations on CMV and HSV suggest no increased incidence or chronicity of these viral infections in CHM and their roles as etiological factors of this disease can be ruled out.

HPV infection is one of the most frequently acquired sexually transmitted diseases and many epidemiological and molecular studies have shown a conclusive link between HPV and human carcinogenesis. It is reported in benign and malignant tumours in a variety of body sites, such as the uterine cervix and anogenital tracts (Arends et al., 1990). The authors noticed HPV staining in the cytotrophoblasts and syncytiotrophoblasts of both normal placentae and CHM. An interesting finding was that the percentage of positive cases was considerably higher in CHM (Table 1). The staining was generally negative to mild in normal placentae (Fig. 1 E). 13.5% of CHM showed intense staining (Fig. 1 F). The difference in mean staining scores was also statistically significant (Table 1).

The immunohistochemical findings of HPV were confirmed by PCR. About 80% of the samples showed positivity in immunohistochemistry as well as in PCR (Fig. 1 G). The samples that showed intense staining in immunohistochemistry gave moderate to intense bands in PCR also.

The ELISA titres of HSV also did not show much difference in the serum of normal placentae and CHM. About 12% the normal placentae were positive while among the CHM 15% were positive. The mean titre was slightly higher in CHM (1.65 ± 0.17 in normal placenta vs 2.32 ± 0.15 in CHM).

In conclusion, HPV infection appears to be significantly associated with CHM while HIV, CMV or HSV showed no association with the pathogenesis of this disease. The only other study on association of HPV with this disease (Pao et al., 1995), reports HPV positivity in about 18% of hydatidiform moles and 50% of choriocarcinomas. Present study supports this observation with HPV positivity in 31% of CHM (confirmed by PCR). This is the first report of an association of HPV with CHM from India.

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References


