SUMMARY & CONCLUSIONS
Gestational trophoblastic disease includes a spectrum of diseases from the potentially pre-malignant hydatidiform mole to the highly aggressive choriocarcinoma. It is a manifestation of an abnormal conception and its incidence is comparatively high in this part of the world. The first line treatment of molar pregnancies is suction evacuation followed by β-hCG follow up to assess the disease progression. Although many advancements have been made in its treatment strategies, the molecular alterations in this disease still remains an etiologic enigma and the biology and oncogenic status of this disease is not very well understood.

The growth of the normal placenta as such can be considered as pseudomalignant and the hydatidiform moles are comparatively more aggressive lesions. The main cause of cancer and its progression is considered to be alterations at the genetic level. Hence, in the present study the different molecular aspects leading a cell to malignancy such as the expression of oncogenes, tumour suppressor genes, proliferative status, expression of apoptosis related genes, metastasis associated genes and viral proteins in the pathogenesis and progression of complete hydatidiform moles (CHM) were studied in comparison to gestational age matched normal placentae.

Immunohistochemical evaluation was carried out in paraffin embedded sections using monoclonal/polyclonal antibodies. The oncogenes studied include the growth factors, EGF and TGF-α, growth factor receptors, EGFR and c-erbB-2 and signal transducers ras and c-myc. The tumour suppressor protein alterations studied were that of Rb, p53 and p21. The proliferative status of the tissue was studied using
antibodies to PCNA and Ki-67. Aberrant expression of the apoptosis related proteins bcl-2 and caspase-3 and that of the metastasis related proteins E-cadherin, P-cadherin, CD44, CD44v6 and nm23 was also looked into. The protein expressions were correlated with the prognosis of the disease using univariate analysis to see if they can be used as indicators of persistence/invasiveness of the disease. The gestational age of presentation of the disease was also correlated with prognosis. On analysing the prognostic value of the biomarkers in this disease emphasis was given at a practical approach to markers which can preferentially be applied to tissue sections rather than involving other modalities of investigation which may require specialised equipment and technology. The parameters that showed significance in univariate analysis were analysed by multivariate analysis to ascertain their role as independent prognostic indicators. A possible association of the pregnancy related viruses like human immunodeficiency virus (HIV), cytomegalo virus (CMV), herpes simplex virus (HSV) and human papilloma virus (HPV) with the etiology of the disease was looked into using immunohistochemical/ELISA/PCR techniques. VDRL test was carried out to look into the association of syphilis with this disease. The β-hCG concentration in serum was also determined using ELISA to determine the progression of the disease. Another aspect of the study was to have a correlative analysis of the different proteins to study their interactions in normal placentae and molar placentae.
The salient findings of the study are given below:-

1. Molar placentae of early gestational ages showed poorer prognosis compared to those of late gestation.

2. There was significant overexpression of EGF, TGF-α, EGFR and c-erbB-2 in CHM compared to normal placentae.

3. In univariate analysis, EGF, TGF-α, EGFR and c-erbB-2 were related to prognosis of the disease and EGF and EGFR to invasion.

4. The cytoplasmic effector molecule rasp21 was overexpressed in molar placentae.

5. In univariate analysis, the tumour suppressor proteins viz., Rb and p53 showed a reduction in the invasive group. This is an indication suggesting a downregulation of these tumour suppressor proteins during invasion.

6. There was significant overexpression of PCNA and Ki-67 in the molar placentae indicating the hyperproliferative nature of the disease.

7. PCNA and Ki-67 showed a significant increase in the persisting disease group and chemotherapy group of patients respectively and both these markers showed relation with invasion in univariate analysis.

8. The apoptosis related gene caspase-3 expression was significantly altered in the molar placentae, suggesting a possible impairment in apoptosis. bcl-2 expression was also significantly altered in the invasive group of patients in univariate analysis.

9. Among the metastasis related proteins studied, CD44 expression was altered in molar placentae and in univariate analysis nm23 showed a reduction in the invasive lesions.
10. Among the markers previously listed, we found that TGF-α and gestational age showed high statistical significance with prognosis in multivariate analysis also, indicating the persistence of the disease.

11. Decrease in Rb expression as well as increased Ki-67 expression emerged as independent prognostic indicators of invasion in multivariate analysis. Thus, tumours with invasive nature can be identified during initial diagnosis, which has consequences for therapeutic management of the patients.

12. Among the genital viruses studied, HPV showed a higher incidence in the molar placentae compared to the normal placentae.

13. A lack of association of syphilis infection with the etiology of CHM was evident.

14. The simultaneous overexpression of growth factors and receptors and their significant relations in bivariate analysis suggest autocrine mechanism of action of these growth factors in these tumours.

15. The highly significant overexpression of TGF-α in molar placentae, its relation with EGFR and also its relation with persistence of the disease in multivariate analysis suggest that TGF-α may play a more active role compared to EGF in the trophoblastic tissue.

16. An increased expression of c-erbB-2 in the molar pregnancies and its relation with EGF and TGF-α in these tumours by bivariate analysis indicate that EGFR may play a transactivating mechanism in these tumours through c-erbB-2 and the overexpression of c-erbB-2 may contribute to increased ligand binding affinity of the EGFR and thereby an increase in the proliferative capacity.

17. The association of the growth factors and receptors with the cytoplasmic
effector molecules in bivariate analysis also implicate their mitogenic roles. Growth factor and growth factor receptor alterations mainly concerning signal transducing systems seem to reflect increased disease aggressiveness.

18. Both in normal placentae and hydatidiform moles, p53 expression showed correlation with the expression of Rb, ras, p53, bcl-2, PCNA and Ki-67. This suggests a possible relation between nm23 and the proliferative function of the cell.

19. Lack of association between p53 and p21 in bivariate analysis suggest p53 independent pathways of p21 activation in the trophoblastic tissue.

Correlative analysis of the different genes in normal and molar placentae indicates drastic differences in the two tissues. Our results suggest that progression of complete hydatidiform mole is a consequence of more than one genetic lesion and suggest that activation of growth factor autocrine mechanisms play a role in the multi-step process of tumour invasion perhaps serving as an initiation event, followed by deregulation of tumour suppressor genes. Future efforts should be directed to study the use of the significant parameters in clinical practice and at the basic level, involvement of these oncoproteins and tumour suppressor proteins in these tissues at the genetic and functional levels.