Chapter 7

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7.1 Study rationale:

Cervical cancer is the second most common cancer among women in many developing countries, including India (1). Though cervical cancer is multifactorial in origin, the genetic predisposition also plays an important role. Although HPV is considered as a major causative agent of cervical cancer, yet the viral infection alone is not solely responsible for cancer progression and/or malignancy. Identification of genetic variants that are associated with cervical cancer will enhance our understanding of the cellular and molecular mechanisms involved in the etiopathogenesis of cervical cancer. The clinical sequelae in cervical cancer progression involve a complex interplay of diverse candidate genes in biochemical pathways in the host that may act as plausible biomarkers for risk assessment of cervical cancer.

Targeting immune-related and/or inflammatory pathways such as the Toll-like Receptor and Cyclooxygenase-2 signaling pathways appears to have immense therapeutic potential in recent times. Single nucleotide polymorphisms in TLR and COX-2 genes have considerable role in disease susceptibility, including cancer. The present study, therefore, aimed to identify the role of TLR and COX-2 gene polymorphisms in cervical cancer susceptibility in North Indian women. Further, the possibility of association of TLR and COX-2 gene polymorphisms in cancer stages and modulation of cancer due to tobacco usage were also investigated.

7.2 Methodology and Overall findings:

To the best of our knowledge, this is the first study exploring the role of TLR and COX-2 gene polymorphisms in the risk of developing cervical cancer in North Indian women. In the present case-control study, a total of 400 study subjects viz. 200 cervical cancer cases and 200 healthy controls were recruited during a two-year period (December 2007-November 2009). Peripheral blood samples were collected from histopathologically confirmed cervical cancer patients from North India and 200 unrelated, cancer-free, age-matched healthy female controls of similar ethnicity. Genomic DNA was extracted using the salting-out method, and genotyped for TLR and COX-2 gene polymorphisms using polymerase chain reaction-based restriction fragment length polymorphism (PCR-RFLP).

The findings did not reveal any significant association between TLR 2 (-196 to -174 del), TLR 3 (c.1377C/T) [rs 3775290], TLR 4 (Asp299Gly) [rs 4986790], TLR 4 (Thr399Ile) [rs 4986791] and
TLR 9 (G2848A) [rs 352140] gene polymorphisms and cervical cancer susceptibility. However, TLR 3 CT+TT was marginally associated (P=0.061; age-adjusted OR=1.46; 95% CI=0.98-2.16) with cervical cancer susceptibility in the study population. Further, no significant association was observed in COX-2 -765G/C [rs 20417] gene polymorphism. Interestingly, the CC genotype of COX-2 +8473T/C [rs 5275] gene polymorphism showed an inverse association with the development of cervical cancer, thus suggesting a possible protective effect (P=0.036; OR=0.35; 95% CI=0.13-0.93). There was no significant association between TLR 2, 3 and 4 gene polymorphisms and cancer stages. The GA genotype of TLR 9 showed borderline association (P=0.056, OR=0.31, 95% CI=0.09-1.03) with stage II of cervical cancer. However, no significant association was observed between TLR 9 polymorphism and FIGO stages III and IV. Moreover, TLR 9 AA genotype showed marginal association (P=0.053, OR=2.63, 95% CI=0.99-7.01) with advanced stages of cervical cancer. The data did not demonstrate any significant association of COX-2 polymorphisms with clinical stages of cervical cancer. Furthermore, no significant association between TLR and COX-2 gene polymorphisms and modulation of cervical cancer risk due to tobacco usage was observed. During the course of the present study, the emerging significance of HPV genotyping was realized and preliminary findings suggested that higher the viral load in terms of test cut-off value, higher was the susceptibility to develop cervical cancer.

A schematic depiction of the overall findings of the present study has been presented in Figure 7.2.1.

Figure 7.2.1. Schematic representation of the overall findings of the present study.
7.3

Conclusions:

To the best of our knowledge, the present study provides the first evidence of the emerging role of TLR and COX-2 gene polymorphisms in cervical cancer susceptibility in North Indian women. Our findings demonstrated that TLR 3 (c.1377C/T), TLR 9 (G2848A) and COX-2 (+8473T/C) gene polymorphisms may have a significant impact on the risk of developing cervical cancer in our study population. However, COX-2 +8473T/C [rs 5275] gene polymorphism showed an inverse association with the development of cervical cancer, thereby suggesting a possible protective effect (P=0.036; OR=0.35; 95% CI=0.13-0.93). Thus, TLR and COX-2 gene polymorphisms, upon further evaluation, may be helpful in elucidation of immunobiological mechanisms associated with cervical cancer susceptibility.

The present study is likely to advance the existing knowledge in disease pathophysiology and public health by providing mechanistic insights for design of immunotherapeutic vaccines against HPV and treatment of cervical cancer in the near future. Moreover, the findings of our exploratory study may be beneficial in cervical cancer management in the near future by providing insights into the identification of clinically-relevant TLR agonists and/or antagonists and selective COX-2 inhibitors in cervical cancer immunotherapy. Nevertheless, further studies in larger cohorts are required to explore the role of TLR and COX-2 gene polymorphisms in the risk of developing cervical cancer, especially in ethnically disparate populations.

7.4

Future perspectives:

We have used candidate gene approaches for the case-control association studies. However, recent advances in human genome research and accompanying technologies have made it possible to explore genetic variations in the entire human genome. These genome-wide association studies (GWAS) utilize high density microarray technologies and complex statistical tools to derive significant associations. Although these studies are costly, yet the information generated can be many magnitude larger than candidate gene approaches. Therefore, in the near future, application of GWAS along with functional genomics approaches in cervical cancer is likely to engender innovative role of genetic factors and their interactions with the environment.