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Expression of miRNA 335 in Gall Bladder Cancer: A Correlative Study

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Abstract : Introduction: Carcinoma gallbladder is third most common gastrointestinal lethal disease with the highest incidence and mortality rate among women in Northern India. Scientists have found several risk factors that make a person more likely to develop gallbladder cancer; among these risk factors, deregulation of miRNAs has been demonstrated to be one of the most crucial factors. The changes in the expression of specific miRNA genes result in the control of inflammation, cell cycle regulation, stress response, proliferation, differentiation, apoptosis and invasion thus mediate the process in tumorgenesis. The aim of this study was to investigate the role of MiRNA-335 and may as a molecular marker in early detection of gallbladder cancer in suspected cases. Material and Methods: A total of 20 consecutive patients with gallbladder cancer aged between 30-75 years were registered for the study. Total RNA was extracted from tissue by using the mirVANA MiRNA isolation Kit according to the manufacturer's protocol. The MiRNA- 335 and U6 snRNA-specific cDNA were reverse-transcribed from total RNA using Taqman microRNA reverse-transcription kit according to the manufacturer's protocol. TaqMan MiRNA probes hsa-miR-335 and Taqman Master Mix without AmpEase UNG, Individual real-time PCR assays were performed in a 20 µL reaction volume on a Real-Time PCR system (Applied Biosystems StepOnePlus™) to detect MiRNA-335 expression in tissue. Relative quantification of target MiRNA expression was evaluated using the comparative cycle threshold (CT) method. The correlation was done in between cycle threshold (CT Value) of target MiRNA in gallbladder cancer with respect to non-cancerous Cholelithiasis gallbladder. Each sample was examined in triplicate. The Newman-Keuls Multiple Comparison Test was used to determine the expression of miR-335. Results: MiRNA335 was found to be significantly downregulated in the gallbladder cancer tissue ($P<0.001$), when compared with non-cancerous Cholelithiasis gallbladder cases. Out of 20 cases, 75% showed reduced expression of MiRNA335, were at last stage of disease with low overall survival rate and remaining 25% were showed up-regulated expression of MiRNA335 with high survival rate. Conclusion: The present study showed that reduced expression of MiRNA335 is associated with the advancement of the disease, and its deregulation may provide important clues to understanding it as a prognostic marker and opportunities for future research.

Keywords : carcinoma gallbladder, downregulation, MiRNA-335, RT-PCR assay
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