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Abstract

Nasopharyngeal carcinoma (NPC) is a unique epithelial malignancy triggered by Epstein Barr virus that occurs at a high frequency in certain regions of southeast Asia. Previous studies reported that this tumor was a radiosensitive but the predominant failure after adequate radiotherapy was due to its distant metastasis. It would be of value to detect the presence of metastasis as early as possible. Previous studies have shown that cytokeratin 19 (Ck 19) positive cells indicate the presence of micrometastasis. The purpose of this study was to evaluate the presence of Ck 19 mRNA by nested RT-PCR in established NPC patients and its correlation to the various stages of NPC as determined by conventional clinical examination. The reported sensitivity of RT-PCR was around 1/10^7, i.e one cancer cell can be detected among 10^7 normal blood cells. Venous blood samples from 30 patients with biopsy-proven nasopharyngeal carcinoma and 10 ENT non NPC patients were tested. After we isolated total mRNA with Trizol reagent, the procedure was continued with nested RT-PCR. The primer sets were directed to conserved regions of Ck 19 genome encoding cytokeratin protein (outer primers: Ck 19-1 & Ck 19-2 and inner primers: Ck 19-3 & Ck 19-4). A distinct 518 bp band (it cannot be seen on agarose gel electrophoresis) and 371 bp of the PCR products indicated the presence of mRNA Ck 19. Results showed that Ck 19 mRNA was obtained in 22 (73%) of 25 NPC patients with several stages and none of 10 (0%) ENT non NPC patients were tested. After we isolated total mRNA with Trizol reagent, the procedure was continued with nested RT-PCR. The primer sets were directed to conserved regions of Ck 19 genome encoding cytokeratin protein (outer primers: Ck 19-1 & Ck 19-2 and inner primers: Ck 19-3 & Ck 19-4). A distinct 518 bp band (it cannot be seen on agarose gel electrophoresis) and 371 bp of the PCR products indicated the presence of mRNA Ck 19. Results showed that Ck 19 mRNA was obtained in 22 (73%) of 25 NPC patients with several stages and none of 10 (0%) ENT non NPC patients. We divided 30 NPC patients into 4 groups according to TNM system (WHO criteria): Stage I: none, Stage IIa : 1 patient (Ck 19 mRNA was negative), Stage IIb: 1 patient (Ck 19 mRNA was negative), Stage III: 3 patients from 5 patients had Ck 19 mRNA positive (60%), Stage IVA: 5 patients from 7 had Ck 19 mRNA positive (72%) and Stage IVb: 14 patients from 16 had Ck 19 mRNA positive (87.5%). No Ck 19 positive cells were detected in control group, 10 patients ENT non NPC (0%), i.e 100% specificity. Our data indicated that the positive detection rate for Ck 19 mRNA in peripheral blood increased as the clinical stage of tumor increased, and the correlation was strong with statistical significance of r = 0.95 and t > 0.02. In conclusion, this study shows that the presence of Ck 19 mRNA, as detected by RT-PCR in NPC, is only significantly associated with micrometastasis tumor. It is a highly specific and sensitive method. Ck 19 mRNA detection is a useful and reliable method to monitor local recurrence and tumor metastasis in NPC patients. It helps to detect recurrence and tumor metastasis early and may improve local and systemic control and enhance patient's survival.

Keyword : Nasopharyngeal, Carcinoma, Ck, 19, mRNA, , nested, RT-PCR, , micrometastatic, tumor,

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