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QUANTITATIVE REAL-TIME PCR EVALUATION OF FREE-CIRCULATING DNA IN PLASMA OF PATIENTS WITH RESECTABLE NON-SMALL CELL LUNG CANCER: PRELIMINARY RESEARCH

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Objective: Many reports of the last decade suggest that cell-free plasma DNA provides useful genetic biomarkers for early diagnostics and clinical outcome prediction in lung cancer patients. Thus, the choice of reliable and efficient method of plasma DNA quantification and its capacity to distinguish between health and cancer would be an essential step prior to any clinical evaluation of cell-free DNA measurement. Apart from the mean plasma DNA content determination, real-time qPCR enables rapid and convenient circulating DNA fragment-length assessment. Recent papers demonstrate the level of plasma DNA integrity may reflect the clinical state of cancer patient and possibly become a tool in prognosis and follow-up monitoring. The aim of present preliminary study was the quantitative analysis of DNA content in plasma samples from healthy volunteers and non-small cell lung cancer (NSCLC) patients before and after surgery. **Methods:** blood samples were collected from 16 healthy volunteers and 14 NSCLC patients (IA-IIIB) prior to and within one week after surgery. The extracted plasma DNA was measured quantitatively by real-time qPCR using β-actin gene as the amplifying target, likely present in all normal and neoplastic cells. The DNA integrity index, defined as the ratio in relative abundance of 400 versus 100 bp PCR products, was analyzed using one forward primer and two nested reverse primers for long and short amplicons. **Results:** the Kruskal-Wallis ANOVA analysis showed a significant differences in plasma DNA content between healthy volunteers (2.65 ng/ml) and NSCLC patients before (12.10 ng/ml) and after surgery (68.74 ng/ml; all $p \leq 0.02$). NSCLC patients presented higher content of 400 bp fragments after the surgery, than before treatment and healthy controls (all $p<0.02$). The plasma DNA integrity index differed significantly between untreated cancer patients (0.15) and healthy control group (0.43; $p=0.004$) and demonstrated a trend towards further decrease in patients after the surgery (0.07; NS). **Conclusions:** Physiological status significantly affects the cell-free DNA content in plasma. The mean plasma DNA content, determined by real-time qPCR, proved to be several-fold higher in cancer patients than healthy volunteers, indeed. Elevated levels of both 100 bp and 400 bp fragments of plasma DNA in cancer patients after surgery may indicate the intensified apoptotic and necrotic processes, mainly due to postoperative trauma. The highest value of DNA integrity index in healthy controls suggests the predominance of apoptotic origin of their plasma DNA. The preliminary results presented need further investigation in a study with larger cancer patient group.