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Serum nerve growth factor level indicates therapeutic efficacy of ^{125}I seed implantation in advanced pancreatic adenocarcinoma

J.-B. XU[1], X.-C. YANG[1], J.-H. GUO[1], J.-Y. CHEN[2], Y. SHANG[1], W.-J. LIU[1], T.-M. WANG[1], L. ZOU[1]

[1]Department of Second General Surgery, The First People's Hospital of Yunnan Province, The Affiliated Hospital of Kunming University of Science and Technology, Kunming, P.R. China
[2]The Affiliated Second Hospital of Kunming Medical University, Kunming, P.R. China

Abstract. – OBJECTIVE: To evaluate serum nerve growth factor (NGF) as a marker in predicting effectiveness of 125I seed implantation in advanced pancreatic carcinoma.

PATIENTS AND METHODS: A total of 45 patients (30 males/15 females with mean age of 52.07±8.43 years) diagnosed with advanced pancreatic adenocarcinoma (PCa) between January 2011 to May 2014 were enrolled as PCa group in this study. Tumors were categorized as at least stage III with unresectionable condition by the TNM standard. The average tumour shortest diameter was 37.54±13.84 mm (18.50-71.20 mm). NGF level in serum before 125I seed implantation and in tumor tissue resected during surgery was measured by ELISA. After treatment, CT Scan was used to serially monitor the diameters of the tumour monthly for 6-month follow-up. RECIST was applied to evaluate the efficacy. Predictive value of serum and tumour derived NGF was evaluated based on ROC curve chart.

RESULTS: We found that the serum NGF level was significantly increased in PCa patients (775.60 ± 250.97 pg/ml) compared to the healthy control group (35.03 ± 25.36 pg/ml), after age and gender adjustment. In the PCa group, the serum NGF level positively correlated with that from loci tumor tissue (r=0.487). The serum NGF level was compared between the effective group (537.42 ± 122.61 pg/ml) and noneffective group (883.17 ± 217.79 pg/ml), and significant difference was detected (p<0.0001). Patients with lower serum NGF level had good response to the 125I seeds implantation. Taking cut-off at 649.59 pg/ml, 85.70% specificity and 90.30% sensitivity were achieved by ROC. Area under the Curve of serum NGF was 0.945, standard deviation was 0.032, 95% confidence interval was 0.882-1.000.

CONCLUSIONS: The level of serum NGF could be a referential index to predict the therapeutic efficacy of 125I seed implantation treatments in advanced pancreatic adenocarcinoma.

Key Words:
Pancreatic adenocarcinoma, 125I seed, Internal radiation, Nerve growth factor (NGF), Biomarker.

Introduction

Pancreatic adenocarcinoma (PCa) is one of the leading diseases with the highest mortality rate in the world. Only 2.8% of new cancer cases were diagnosed as PCa but it accounted for 6.8% of all cancer death[1]. In 2012, more than 70,000 PCa related death was reported in China by WHO. 80% of the patients' tumours were unresectionable due to late stage diagnosis[2]. The traditional treatments for PCa patients include resection, chemo-therapy and radioactive therapy, depending on the grade and stage classification. Despite rapid development of anti-cancer drugs and surgical improvements, the 5-years survival rate still remains relatively low (6.7%) for the past 30 years. 125I seeds implantation is a radioactive regime which involves direct application of iodine 125 particles into the tumour loci to induce cancer cell apoptosis through short-range γ-radiation. This treatment modality has been demonstrated to be suitable for liver, lung, prostate and pancreatic cancer known to be sensitive to radiotherapy[3-6]. 125I seeds implantation has been adopted as an alternative method for treating PCa patients who were not suitable for resection in China since 1981[7]. Several studies indicated the success of 125I implantation in reduction of tumour size, pain relief, enhanced survival and general improvement in quality of life without significant side effects[6-10]. As no

Corresponding Author: Xiaochun Yang, MD; e-mail: blackstone502@163.com
Jianhui Guo, MM; e-mail: 291600738@ qq.com

long-term survival benefit from this treatment[11] set with a prohibitive cost, one major motivation is to minimise exposure of non-responders to this treatment.

Nerve growth factor (NGF) which belongs to the nerve growth factor family of neurotrophin is implicated in angiogenesis and the survival and proliferation of cancer cells, culminating in tumour progression and metastasis[12]. NGF in PCa tissues has been shown to have strong correlation with tumor proliferation, encroachment and migration[13]. Although sampling tissue NGF remains technically infeasible, NGF is secreted from loci tissue into blood circulation system, thus serum NGF could act as a proxy for tissue derived NGF. Mirroring this, Liss's study[14] reported that the postage cancer grades can be classified by measuring urine NGF levels. Hence in an effort to investigate the value of serum NGF as a prognostication tool for determining clinically favourable outcomes in advance PCa patients receiving ^{125}I treatment, we measured the serum NGF levels in advanced pancreatic cancer patients who are withdrawn from traditional resection and are on brachytherapy of ^{125}I.

Patients and Methods

Patients and Specimens Selection

Between Jan 2011 and May 2014, 54 advanced pancreatic cancer patients who are diagnosed histologically and received ^{125}I seed implantation as treatment at the First People's Hospital of Yunnan Province were selected for this study (PCa group). Frozen section during surgery and pathological examination after surgery confirmed all enrolled patients had at least stage III PCa. Key exclusion criteria were grade less than stage III; history of coagulopathy, hypertension and stroke or transient ischemic attack; co-morbidities such as severe heart, lung, liver, kidney or encephalon diseases; distant metastasis; a follow-up period of fewer than 6 months. Protocol was reviewed and approved by the First People's Hospital of Yunnan Province ethics committee. Patients provided written informed consent before study entry. In addition, we recruited 50 healthy adult subjects as a control group (HC group).

Serum NGF Level test

The venous blood of each subject was taken 1 week before treatment and centrifuged for serum separation. NGF level in serum was measured using Euzymelinked Immunosorbent Assay (ELISA) kit (Merck Millipore, Temecula, CA; USA). The data was read by ELISA meter (BioTek Instruments, Inc., Winooski, VT, USA).

Tissue NGF level test

Fresh tumor tissues from PCa patients biopsied during surgery were homogenized in RIPA lysis buffer (1% NP-40, 50 mM Tris-Hcl, PH 7.4, 150 mM Nacl, 1% sodium-deoxycholate, 0.1% SDS, 0.5 m M EDTA, PH 8.0, 1 mM PMSF) for 30 min in ice, than centrifuged at 25,000 ×g for 30 min at 4°C. Equal amount of protein was determined using BCA assay (Pierce, Pittsburgh, PA, USA). Protein sample was measured by ELISA. The kit bought from Merck Millipore Company (Temecula, CA, USA).

Computed Tomography (CT Scan)

CT scan (SOMATOM Definition AS, Siemens, Amberg, Germany) was performed before surgery with a lamella thickness of 2 mm. The shortest diameters of tumor were measured. Computer stereotaxis plan system (Prowess 3D Version 3.02, SSGI, Inc., Orlando, FL, USA) was used to design quantities and activity of 125I seed. CT was applied for following up every month after surgery till up to 6 months.

125I seeds Implantation

During the surgery, pancreatic tumor was fully exposed. Sources were inserted at interval of 1-1.5 cm with activity series $(2.2\text{-}3.3)\times10^7$ Mq seeds (GMS Pharmaceutical Co., Ltd, Shanghai, China). The average number of seeds that were implanted was 49±11.59 for each patient (25-75 seeds).

Definition of Efficacy

The results from 6 months after the surgery were adopted and evaluated by the standard of new response evaluation criteria in solid tumours (RECIST). Complete remission (CR), partial remission (PR) and stabilization (SD) were considered effective, process aggravation (PA) was considered ineffective.

Statistical Analysis

Baseline characteristics of all the patients were collected and were analyzed with IBM SPSS statistics 19.0 software (IBM SPSS Inc., Chicago, IL, USA). Data was presented as mean ± SD. *T*-test was used for significant difference testing of

continuous variables. Pearson correlation was used to determine the connection of variables. Specificity and sensitivity were determined by receiver operating characteristic (ROC) curve. p value < 0.05 was set for statistical significance.

Results

Baseline Characteristics

A total of 54 patients were enrolled in the PCa group. However, 6 cases were excluded for death within 3 months after treatment and 3 cases were excluded for distant metastasis. Finally, 45 patients were included in PCa group (Figure 1). The demographics of the patient cohort consisted of 30 males and 15 females with an average age of 52.07±8.43 (41-72) years. MRT/CT indicated a tumour size of 18.50-71.20 mm in average 37.54±13.84 mm in shortest diameter before treatment. In HC group, the average age was 55.23±7.21 (42-75), 32 males and 18 females. There were no statistically significant differences in sex and age between the two groups.

Serum NGF level in PCa group

Serum NGF levels were examined one week before surgery for 45 patients in the PCa group and 50 healthy adults were enrolled as control. The mean value of serum NGF in PCa group was 775.60 ± 250.97 pg/ml, which was about 22 fold higher than that in HC group (Mean=35.03 ± 25.36 pg/ml) (p<0.0001; Figure 2).

Serum NGF Level Indicated Outcome of 125I Seeds Implantation

14 of 45 PCa patients (31.11%) who had good responds to the treatment, based on RECIST standard, were classified as effective group (EG). Among all the 14 cases, 9 cases (20.00%) had PR and 5 (11.11%) had SD. The remaining 31 patients (68.89%) who had PA were categorised into the non-effective group (NEG). Serum NGF levels between these two groups were compared. As showed in Figure 3, patients in EG group had relatively low NGF level (Mean=537.42 ± 122.61 pg/ml), compared to NEG group (Mean=883.17 ± 217.79 pg/ml). There was statistically significant difference in serum NGF level between the two groups (p<0.0001). 90.30% sensitivity and 85.70% specificity could be achieved when point A, which was also reflected in Figure. 3 as red line (649.59 pg/ml), was chosen as cut-off point in ROC (Figure 4). Area under the Curve (AUC) of serum NGF was 0.945, standard deviation was 0.032, 95% confidence interval was 0.882-1.000. In 15 patients with serum NGF level ≤ 649.59 pg/ml, 12 were effective and 3 were ineffective. In another 30 ones with serum NGF level > 649.59 pg/ml, 2 were effective and 28 were ineffective. Positive predictive value was 80.00% (12/15) and negative predictive value 93.33% (28/30).

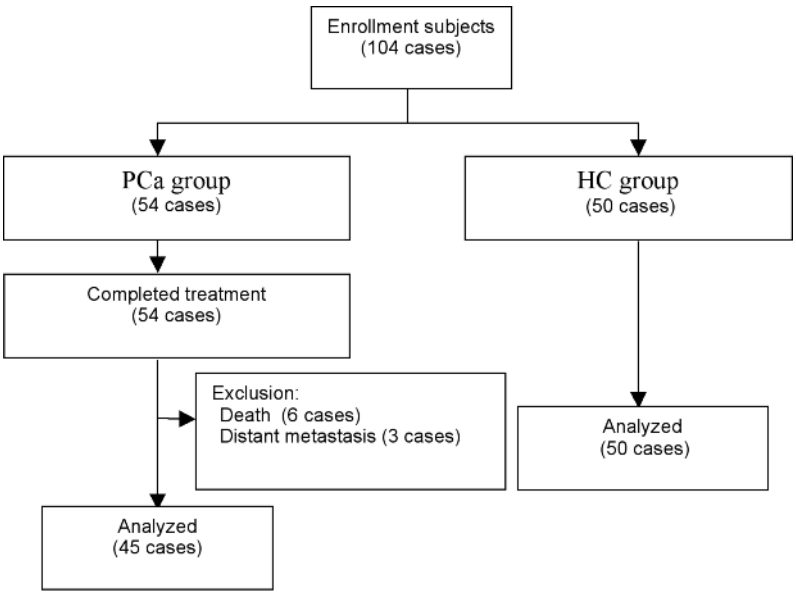

Figure 1. Chart showing participants flow.

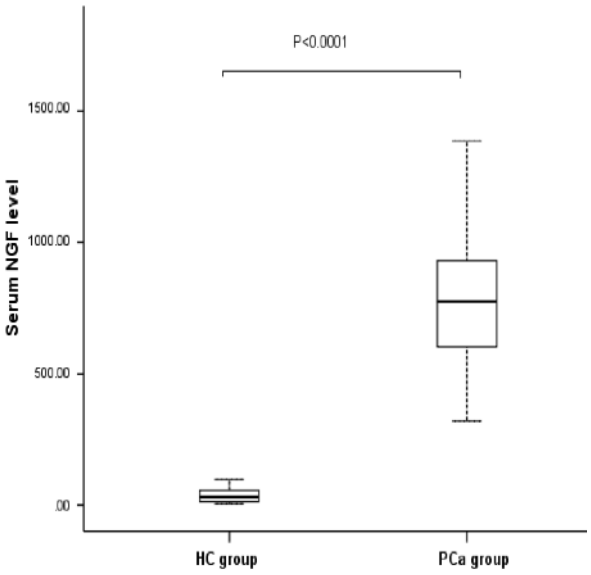

Figure 2. Serum NGF level between HC group and PCa group.

Serum and Tissue NGF Level had Positive Correlation in PCa group

Pre-surgery serum NGF value was compared to the tissue NGF level after surgery. There was good correlation in NGF level between serum and tissue from the same patient (r=0.487, p=0.001; Figure 5).

Discussion

Development of PCa therapeutic agents, even in the initial stage of invasive cases, emphasizes the importance of early detection. However, the complex pathophysiology, absence of early diagnostic and prognostic markers and unresponsiveness to radiation and chemotherapies are major barriers against successful therapy in PCa[15].

It has been confirmed that NGF plays an important role in the growth, development and prognosis of certain types of cancer, such as breast, prostate, liver and lung cancer[12-14]. NGF has been demonstrated to enhance pancreatic cancer cells growth and proliferate[13], and reduction in pain threshold[16]. It has also been shown that, NGF has positive correlation to pancreatic cancer's encroachment, metastasis[17]. The high expression of NGF in tumour tissue has been thought to be a signal of cancer prognosis. NGF is secreted by neurons and peripheral gliocytes. The increased pool of serum NGF in circulation could have been the direct result of secretion from pancreatic tumour itself or indirect response from other organs or tissues. In our study, we tested serum NGF by ELISA. This result showed that serum NGF level was elevated in PCa patients compared to the healthy control group (p<0.0001). Furthermore, we found positive correlation between paired serum and tissue NGF levels from the same subject. This supports the notion that the increased serum NGF level at least partly originates from the loci pancreatic tumour tissue. Also, this implied that serum NGF value alone, or more practically, together with other PCa specific biomarkers, such as CA19.9, could be a marker or marker panel for PCa follow up and predicting prognosis.

125I seeds implantation has several advantages for the insertional PCa patients. In our study cohort, the PCa patient group who had 125I implantation treatment, only 31.11% patients experienced clinically good response to the treatment which is inline with previous report from Jin et al study[11]. To investigate the reasons for this low response rate, retrieve analysis was performed. Surprisingly we find that most of the patients who responded to the treatment has relatively low serum NGF value, compared to those who has no response (p<0.001). If 649.59 pg/ml of serum NGF was taken as cut off (ROC), the sensitivity and specificity were 90.30% and 85.70% respectively. This implied that, instead of testing tissue NGF,

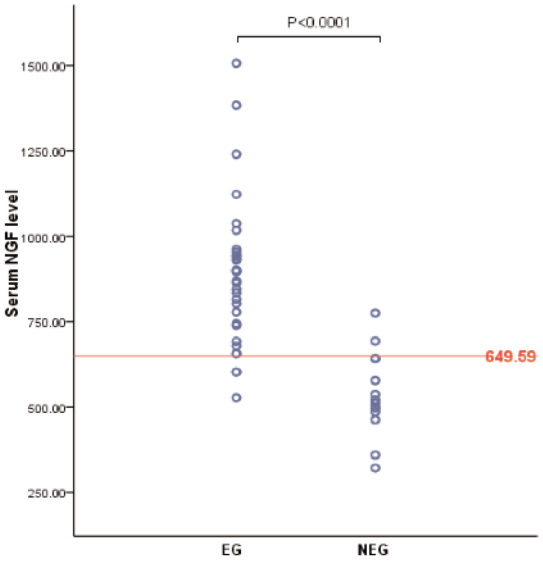

Figure 3. Comparison serum NGF level between effective and noneffective group.

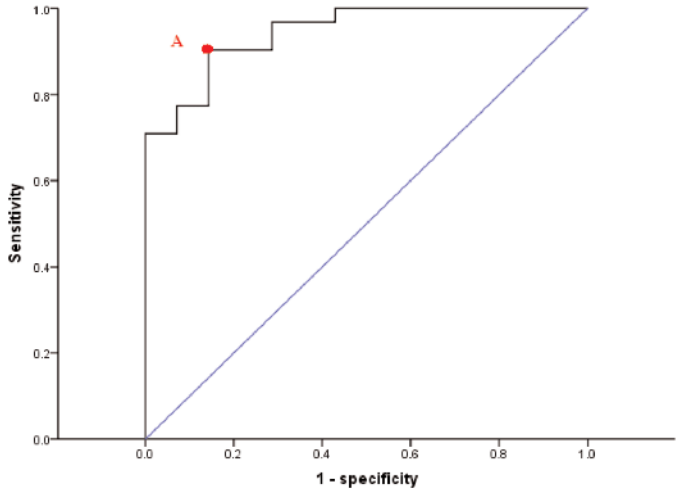

Figure 4. Sensitivity and and specificity by Roc.

which is infeasible, serum NGF level could be an independent predictive biomarker to the efficacy of ^{125}I implantation treatment. Possible reasons that could account for the differences in serum NGF level between EG and NEG include: (a) the differences in the functional state of the tumour in terms of secretion, (b) tumour size, (c) the extent of angiogenesis and (d) the inter-individual clinical status of each patient.

To our best knowledge, this is a novel finding in PCa patients who were treated with ^{125}I seeds implantation. It makes serum NGF as a valuable marker to predict the efficacy of the treatment. It can be used for patient selection and therefore, allow for personalised treatment. Unnecessary economic burden from patients can be reduced and medical resources from hospital side can be saved as well.

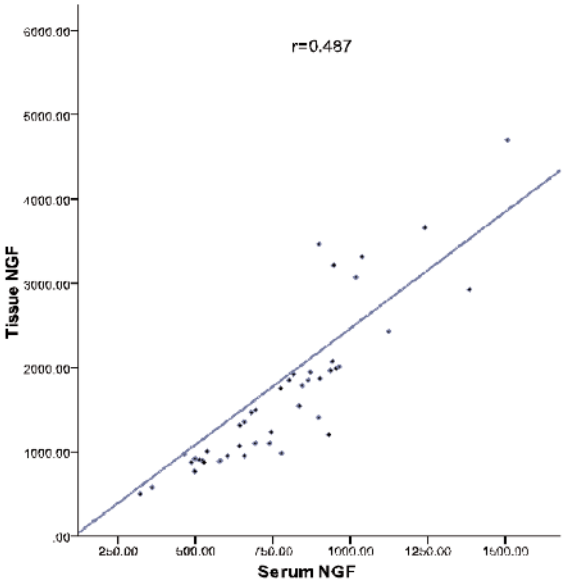

Figure 5. Correlation of serum and tisue NGF level.

Conclusions

We concluded that serum NGF could form as a marker panel for PCa diagnosis and follow up. More importantly, its level predicts the efficacy of ^{125}I seed implantation and then can be a marker for patient selection and personalised treatment. However, larger scale study needs to be done for further verification.

Acknowledgements

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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