Integrative Cancer Science and Therapeutics

Research Article ISSN: 2056-4546

Adrenomedullin in pancreatic carcinoma: A case-control study of 22 patients

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Abstract

Pancreatic carcinoma is a leading cause of cancer-related death. Reduction of the diagnostic delay is mandatory. Adrenomedullin (AM) is overexpressed in pancreatic cancer. A case-control study including 12 patients with pathological diagnosis of pancreatic carcinoma and 10 healthy controls was conducted at our Institution. Blood samples were obtained at the time of hospitalization and post-operatively for cases. Controls' samples were obtained from healthy volunteers. AM was measured by using enzyme immunoassay method. AM showed significant increase in pancreatic carcinoma patients vs controls (4.51 ng/ml vs 1.91 ng/ml, p value = 0.04) regardless of tumor stage, differentiation, resecability/unresecability, diabetes. A cut-off of 1.75 ng/ml reaches a sensibility of 83% and a specificity of 70% (p value = 0.0147; CL 95%; AUC 0.767). The increase of AM didn't correlate with the increase of other common tumor markers (CA 19-9 and CEA), nor direct bilirubin. These data confirm the utility of studying the role of AM in pancreatic cancer, in order to achieve an early diagnosis in high risk populations.

Introduction

Pancreatic carcinoma (PaC) is the fourth leading cause of cancer-related death in the western World [1] and its incidence is increasing in sexes (+ 0.8% per year males; + 2.0% per year females) [2]. Up to now surgical resection is the only curative option for those patients [3]. Overall survival at 5-years for resected patients reaches a 43% rate [4] compared to a global 5-years survival rate of 5% [3]. However, only 20% of patients are diagnosed with a resectable disease, and a complete resection (R0) is achieved in less than 10% [5]. Late clinical signs and absence of specific tests are responsible for the delay in the diagnosis of PaC [3]. Currently, CA 19-9 is the only marker acknowledged worldwide for PaC diagnosis [2,6], and the main limitations to its use are low specificity [6-8] and late increase on disease progression [2]. Adrenomedullin (AM) is up-reguleted in pancreatic cancer [9] and it is investigated as a possible diabetogenic mediator [9]. Both cancer cells and neighboring pancreatic tissue show AM overexpression independently from the stage of the neoplastic disease [9]. The aim of this study was to evaluate serum AM levels with cytological and/or histological diagnosis of PaC compared to non-cancer controls. This represents the validation phase of a larger prospective study that aims to evaluate the role of AM in the early detection of PaC in a high risk population: new-onset diabetic patients [ClinicalTrials.gov Identifier: NCT02456051].

Materials and methods

From November 2013 through August 2014, 16 patients presenting with suspect of pancreatic carcinoma to the Department of General Surgery of St Andrea University Hospital in Rome were evaluated. Patients were enrolled in the study according to the following criteria: pancreatic adenocarcinoma confirmed by cytological or histological sample; written informed consent provided by each patient; age 18-90. Exclusion criteria were: other than ductal adenocarcinoma pancreatic neoplasm; other synchronous malignancies; post operative sepsis or renal failure signs. The main characteristics of the patients are summarized in Table 1. 10 non-cancer volunteers were enrolled in the study as controls, and this population is homogeneous to the cases in terms of age and sex. Their characteristics are summarized in Table 2.

Blood samples were obtained at the time of hospitalization and, when resection was feasible, post-operatively in 7th post-operative day. Each blood sample was centrifuged (4000 RPM for 10 minutes), divided into 1.5 ml aliquots and stored up at -18°C. AM was measured by using commercially available EIA method (Adrenomedullin (Human) EIA Kit, Phoenix Pharmaceuticals, Inc.Mountain View, CA,USA). The intra- and inter-assay coefficients were 5.1% and 12%, respectively.

For statistical analysis Excel program (Microsoft®) has been utilized. T-test was applied to compare serum levels of AM in the study groups. Comparisons were: PaC patients vs non-cancer controls; among the PaC population, earlier stages vs later stages, more differentiated vs less differentiated, resectable vs unresectable, diabetics vs no diabetics; among the operated population, preoperative levels vs postoperative levels. Linear regression and Pearson coefficient were evaluated to detect possible correlation between serum levels of AM and CA 19-9, CEA, direct bilirubin. Correlations between AM and preoperative FPG and AM and diabetes duration were analyzed too.

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Key words: *pancreatic carcinoma; adrenomedullin; diabetes; pancreatic oncomarker*

Received: February 25, 2016; **Accepted:** March 11, 2016; **Published:** March 14, 2016

Table 1. Characteristics of the patients affected by pancreatic adenocarcinoma.

Age –years old- Median (range)	68 (45 – 77)
Sex - No (%) of males	7 (58.3)
Smokers - No (%)	6 (50)
Comorbidities - No (%) - Cardiovascular - Respiratory - Other	 9 (75) 3 (25) 5 (41.7)
ADA criteria for diabetes responding patients- No (%)	6 (50)
Time from diabetes diagnosis to cancer diagnosis –months- Median (range)	66 (1 – 360)
Oncomarkers > reference values No (%) - CA 19-9 - CEA	 7 (58.3) 5 (41.7)
Tumor localization No (%) - Head - Body - tail - Head and body - tail	 10 (83.3) 1 (8.3) 1 (8.3)
Clinical Stage No (%) - I o IA o IB - II o IIA o IIB - III - IV	 1 (8.3) 0 (0) 1 (8.3) 4(33.3) 2 (17) 4 (33.3)
pT[a]- No (%) - T_1 - T_2 - T_3 - T_4	 1 0 5 0
pN[a] - No (%) - N_x - N_0 - N_1	 0 2 4
pM[a] –No (%) - M_o - M_1	 5 1
R – No (%)* - R_0 - R_1 - R_2	 6 0 0
G - No (%)* - G_1 - G_2 - G_3	 3 2 1

[a] pTNM was evaluated in resectable patients (6 of 12)

Table 2. Characteristics of noncancer controls.

Age –years old- Median (range)	55 (25 – 90)
Sex – N. (%) of males	6 (60)
Smokers N. (%)	6 (60)
Diabetes N. (%)	1 (10)
Comorbidities N. (%) - Cardiovascular - Respiratory - Other	 3 (30) 0 (0) 3 (30)

P value was assumed significant when < 0.05. ROC curve for AM was designed to detect the serum cut-off concentration with the best sensitivity and specificity. The study was conducted in the respect of the principles of Helsinki declaration [10].

Results

12 out of 16 patients were included in the study after histological diagnosis of pancreatic adenocarcinoma. The other 4 were excluded for different histological findings. 6 patients underwent a complete tumor resection while the remaining 6 patients received palliative care or chemotherapy regimens. Mean serum AM level in PaC patients at the time of hospitalization was 4.51 ng/ml (range 0.04 – 9.16 ng/ml, SD 3.17). Resected patients had a preoperative mean serum AM level of 4.94 ng/ml(range 0.04 – 9.16 ng/ml, SD 3.56) and a postoperative mean serum AM level was 2.13 ng/ml (range 0.47 – 3.77 ng/ml, SD 1.60). The mean decrease of serum AM was 2.8 ng/ml (range -0.43 – 8.11, SD 3.14) but wasn't statistically significant (p = 0.12). Serum AM was significantly increased in PaC patients than in controls (4.51 ng/ml vs 1.91 ng/ml; p = 0.04) (Table 3 and 4). All other comparisons are shown in Table III. None of the correlations investigated with CA19.9, CEA, bilirubin, FPG and diabetes duration was significant (Table 4 and 5). ROC curve pointed out that a cut-off of 1.75 ng/ml AM had a sensitivity of 83% and a specificity of 70% (p value = 0.0147; CL 95%; AUC 0.767) to distinguish cases from controls (Figure 1).

Discussion

Diagnostic delay is responsible for the high mortality of PaC, which

Table 3. Serum AM levels mean (and Standard Deviation) of the compared groups and respective p value.

	Mean (SD)	Mean (SD)	P value
PaC patients VS Controls	4.51 ng/ml (3.17)	1.91 ng/ml (2.20)	0.04
Stage I-II VS Stage III-IV	5.05 ng/ml (3.97)	4.96 ng/ml (2.51)	0.86
G1 VS G2	6.68 ng/ml (3.86)	2.61ng/ml (3.63)	0.93
Resectable VS Unresectable	4.94 ng/ml (3.56)	4.75 ng/ml (2.80)	0.93
Preoperative VS Postoperative	4.94 ng/ml (3.56)	2.13 ng/ml (1.60)	0.12
Diabetic VS No diabetic	5.45 ng/ml (3.10)	3.57 ng/ml (3.22)	0.33

Table 4. Correlation between serum AM levels and serum CA 19-9, CEA and direct bilirubin levels.

	Pearson coefficient (R^2 and p value)
AM – CA 19-9	0.345 (0.119; >0.2)
AM – CEA	-0.28 (0.08; > 0.5)
AM – Direct Bil.	0.26 (0.07; >0.2)

Table 5. Correlation between serum AM levels and FPG and duration of diabetes.

	Pearson coefficient (R^2 and p value)
AM – FPG	0.28 (0.08; >0.2)
AM – diabetes duration	0.57 (0.33; >0.2)

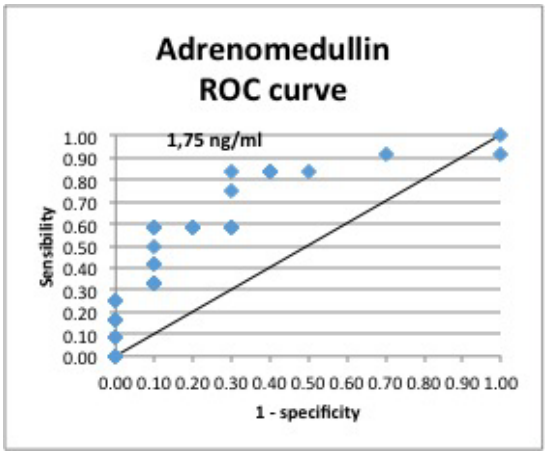

Figure 1. Adrenomedullin (AM) ROC curve. Cut-off 1.75 ng/ml AM (sensitivity 83%, specificity 70%, p val Adrenomedullin ue 0.0147, CL 95%, AUC 0.767).

almost coincides with its incidence [1,3]. PaC-related symptoms are late-onset in the natural history of the disease [3], therefore early serum markers are needed. Unfortunately, the ones currently available are related to late stages, and so are unuseful in detecting a resectable disease [2]. This urge to seek new diagnostic markers led us to investigate the role of Adrenomedullin in PaC. The interest for AM arises from recent studies that investigated PaCDM mediators [12-14]. AM is a 52-amino acid peptide discovered in 1993 [15]. It is physiologically produced by human organism [16] but its secretion increases in multiple pathological conditions, such as endothelial damage, heart failure, hypertension, renal failure, sepsis and hypoxia [17,18]. Physiologically AM is recognized as a regulator of the pancreatic secretion [14,19,20], while in pancreatic cancer, it is overexpressed in both neoplastic and peritumoral tissues. Circulating levels are increased too [9]. Recently new-onset diabetes mellitus has been investigated as a possible early manifestation of pancreatic cancer (PaCDM). 50% of patients affected by PaC are diabetic, 70% of whom were diagnosed for being diabetic less than 2 years before tumor diagnosis [12]. Moreover, PaCDM is an early manifestation of the disease and develops when the tumor is resectable in 55% – 65% of the cases [21]. In our population AM did not show any correlation with DM presence. We might hypothesize that AM is over-expressed in all PaC patients and that only subjects likely to develop diabetes reveal the effects of β-cell dysfunction. In this study we included only pancreatic ductal adenocarcinomas, which is the most frequent histological type of pancreatic carcinoma to avoid confounding factors deriving from heterogeneity of histology [3]. In our population serum AM showed a significant increase in PaC patients but do not correlate with tumor stage, differentiation or resectability. AM levels do not show any relation neither with pre-operative FPG or length of diabetes. Those characteristics could configure AM as an early tumor marker: its stage-independence makes it a promising diagnostic tool. Moreover, AM showed a decreasing trend after resection, even if not in a statistically significant way. We can hypothesize that dosing the peptide later than the 7th pod would have allowed a better washout and, consequently lower AM levels. These preliminary data confirm the tight relationship between Adrenomedullin and Pancreatic Cancer. Further studies are needed to investigate the role of AM as early diagnostic tool in high risk populations.

Acknowledgments

The authors thank Giovanni Clemente and Gerardo Salerno for their support and contribution during the study conduct. Gerardo Salerno also gave his contribution for the statistical analysis.

Disclosure

The presented results represent the validation phase of a larger prospective study that aims to evaluate the role of AM in the early detection of PaC in a high risk population: new-onset diabetic patients [ClinicalTrials.gov Identifier: NCT02456051]. The funds to support this research were provided by Sapienza – University of Rome.

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