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The combined effect of adiponectin and resistin on all-cause mortality in patients with type 2 diabetes: Evidence of synergism with abdominal adiposity

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ABSTRACT

Background and aims: While elevated serum adiponectin and resistin levels have been singly associated with all-cause mortality in patients with type 2 diabetes (T2D), their combined effect has never been studied.
We investigated such joint effect in patients with T2D and its possible modulation by several demographic and clinical conditions, known to affect *per se* mortality rate.
Methods: Patients with T2D from the Gargano Mortality Study (GMS; N = 895, follow-up = 10.5 ± 3.7 years; 290 events) and the Foggia Mortality Study (FMS; N = 519, follow-up = 7.1 ± 2.5 years; 140 events) were examined.
Results: As singly considered, adiponectin and resistin were independently associated with mortality rate in GMS and FMS ($p < 0.0001$ for both). The two studies were then pooled, for investigating the nature of the joint effect of the two adipokines. In such sample, both adipokines were associated with death, independent of each other and of several additional covariates ($p = 0.01–4.58 \times 10^{-12}$). Of note, no adiponectin-by-resistin interaction was observed ($p = 0.40$), thus pointing to an additive effect of the two adipokines. As compared to individuals with low levels of both adiponectin and resistin (i.e. below median values), those with high levels of both adipokines had an HR (95%CI) for death of 3.02 (2.26–4.03). Such increased risk was more pronounced in individuals with relatively low abdominal adiposity (p for HR heterogeneity below or above the median value of waist circumference = 0.03).
Conclusions: Adiponectin and resistin show an additive independent effect on all-cause mortality in patients with T2D. Such effect is modified by abdominal adiposity.

1. Introduction

Type 2 diabetes (T2D) is a leading risk factor for all-cause death, with mortality rate in diabetic patients being twice as much as that in non-diabetic individuals [1]. Great efforts are, therefore, needed to decrease such tremendous burden. A deeper understanding of the role of biomarkers able to predict mortality rate in T2D may help accomplish this goal.

The last decade has witnessed that some adipokines exert an important role in shaping mortality risk [2–6]; among these, are resistin and adiponectin, known to affect intermediate metabolism [7], low-grade inflammation and atherosclerotic processes [8–10]. Resistin is positively related with all-cause mortality in several sets [6,11], including T2D [6,12]. Quite unexpectedly, a similar positive

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association across several conditions [3,4,13–18], comprising T2D [19,20], has been paradoxically reported also for adiponectin. Despite so many data on each of the two adipokines, their combined effect on mortality rate has been investigated only once in a small study, carried out in the specific subset of dialysis patients [21].

Our aim was to explore the interwoven effect of adiponectin and resistin on mortality rate in patients with T2D. In addition, the role of several possible modifiers on such joint effect was investigated.

To pursue this goal, data from over 1400 Italian diabetic patients followed over time for several years were analyzed.

2. Materials and methods

2.1. The Gargano Mortality Study (GMS)

This cohort comprises 1028 patients with T2D (according to ADA 2003 indications) consecutively enrolled from November 1st, 2000 to September 30th, 2005 at the Endocrine Unit of IRCCS "Casa Sollievo della Sofferenza" in San Giovanni Rotondo (Apulia, central-southern Italy), in a study on all-cause mortality [12,22–24]. Poor life expectancy, due to non diabetes-related disorders, was the only exclusion criterion. GMS has been followed-up until 31st December 2014. Vital status was ascertained by telephone interview with patients or their relatives. Alternatively, needed information was obtained through the registry office of residence cities. The last follow-up was carried out by inquiries to the Italian Health Card (http://sistemats1.sanita.finanze.it/wps/portal/portalets/cittadinots/ts).

Serum total adiponectin and resistin were assessed in 895 participants (87.1%) constituting the eligible sample for the present analysis.

2.2. The Foggia Mortality Study (FMS)

This cohort comprises 1253 patients with T2D (ADA 2003) consecutively recruited at the Endocrine Unit of the University of Foggia (Apulia, central-southern Italy) from 7th January 2002 to 30th September 2008 for a study whose end-point was all-cause mortality [23,25].

Also in this case, poor life expectancy due to malignancies was the only exclusion criterion. FMS was followed until 31st March 2015. At follow-up, vital status of study patients was ascertained by telephone interviews or queries to the registry office of residence cities.

Serum total adiponectin and resistin were measured in 519 patients (45.0%), who constituted the suitable sample for the current analysis.

2.3. Examination at baseline

In both studies, clinical data were obtained at baseline from a standardized interview and examination and blood samples were collected in the morning after an overnight fast. Serum aliquots were stored at −80 °C.

Smoking habits, anti-hypertensive, anti-dyslipidemic, and glucose-lowering treatments were registered at the time of examination. No thiazolidinediones were ever used in these patients. Medical registers confirmed data concerning medications. Current smokers were patients smoking cigarettes habitually during the year before the examination. Diabetes duration was calculated subtracting from the current age the age at diabetes diagnosis.

Urinary albumin and creatinine measurements at baseline were determined as previously described [26].

The CKD-EPI formula was used in order to estimate eGFR [27].

2.4. Ethics

Institutional Ethic Committee of Istituto di Ricovero e Cura a Carattere Scientifico (IRCCS) "Casa Sollievo della Sofferenza" and the University of Foggia, respectively authorized both study protocols and informed consent procedures. All participants gave a written informed consent.

2.5. Measurement of circulating adiponectin and resistin levels

Adiponectin and resistin serum concentrations were measured at the Research Unit of Diabetes and Endocrine Diseases at "Casa Sollievo della Sofferenza" by commercial ELISA kits (Alpco, Salem, NH and Bio Vendor, Brno Czech Republic respectively), as previously described [28,29]. Adiponectin and resistin inter- and intra-assay coefficients of variation were 7.0 and 6.6%, and 7.0% and 5.2%, respectively.

2.6. Statistical methods

Baseline characteristics of patients are shown as mean ± SD and percentages for continuous and categorical variables, respectively.

The relationship between either adiponectin or resistin serum concentrations and all-cause mortality was log linear, as assessed by the Kolmogorov-type supremum test based on a sample of 10,000 simulated residual patterns [30]. Consequently, both adiponectin and resistin were analyzed after natural log transformation.

In both GMS and FMS, the time variable was defined as the time between the baseline examination and date of death or of the last clinical follow-up for survivors. Incidence rates (IR) for all-cause death were reported as the number of new events per 100 person years (py). The association between adiponectin or/and resistin and the event occurrence was assessed by univariate and multivariable Cox proportional hazards regressions analyses. Risks were reported as HRs along with their 95% CI for SD increase in natural logarithm of adiponectin or/and resistin levels.

Pooled analyses were performed in an individual data meta-analysis fashion [31], adjusting for "study sample", after testing for heterogeneity (i.e. the presence or absence of a significant exposure-by-sample interaction).

We evaluated six separate models, including several covariates possibly related to the exposures and/or the outcome of our study design. The first model was adjusted only for "study sample" (i.e. GMS and FMS); in the second model sex, age at recruitment and smoking habits were added to the previous model; in the third model BMI and waist circumference were added to the second model; in the fourth model disease duration and HbA1c and age at diabetes diagnosis were added to the third model (in this case, because of its collinearity with diabetes duration, age at recruitment was not included and was replaced by age at diagnosis; in this way the sum of the effect provided by both age at diagnosis and diabetes duration, mathematically correspond to the overall effect provided by the age at recruitment itself); in the fifth model ACR and eGFR were added to the third model; finally, in the sixth model glucose-lowering, anti-hypertensive and anti-dyslipidemic treatments were added to the third model.

Lastly, as possible modifiers of the joint effect of adiponectin and resistin on mortality rate, age at recruitment, sex, smoking habits, BMI, waist circumference, HbA1c, diabetes duration, ACR, eGFR and medications, were investigated. This was tested by Cox proportional hazards analyses stratified according to the above-mentioned variables along with the presence of multiplicative interaction terms.

We considered as statistically significant a p-value <0.05. SPSS