A prospective study of circulating adipokine levels and risk of multiple myeloma

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Short title (running head): Circulating adipokines and multiple myeloma

Abstract

It has been hypothesized that the observed excess risk of multiple myeloma (MM) among obese individuals could be due to altered circulating levels of adipokines, polypeptide hormones with pro- and anti-inflammatory properties secreted by adipose tissue. We investigated whether circulating levels of leptin, total adiponectin, and high-molecular-weight (HMW) adiponectin are associated with subsequent MM risk among 174 MM patients and 348 controls within the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial. Inverse associations with MM were observed for total adiponectin (highest quartile vs. lowest: odds ratio=0.49, 95% confidence interval=0.26-0.93, P_{trend}=0.03) and HMW adiponectin (0.44, 0.23-0.85, P_{trend}=0.01). These associations remained after restricting to MM patients diagnosed approximately eight years or more after blood collection. Leptin levels were not associated with MM risk. The results of this study, to our knowledge the first prospective investigation of circulating adipokines and MM, suggest that adiponectin may play an important role in obesity-related myelomagenesis.

Introduction

Multiple myeloma (MM) is a fatal plasma cell malignancy that will account for approximately 21,700 new cancer diagnoses in the United States (U.S.) in 2012.[1] Recent studies have shown that MM is consistently preceded by monoclonal gammopathy of undetermined significance (MGUS).[2] Established MM risk factors include older age, male sex, African ancestry, family history of MM or MGUS, and severe immune dysregulation.[3,4] Obesity has also been associated with an increased risk of MGUS and MM,[5,6] although the specific biologic mechanisms have yet to be elucidated. Alterations in circulating levels of adipokines, polypeptide hormones secreted by adipose tissue, have been proposed as a potential mechanism through which obesity contributes to myelomagenesis. The most abundant adipokine, adiponectin, is mainly produced by visceral adipose tissue;[7] it has important anti-inflammatory and insulin-sensitizing properties, and circulating levels are negatively correlated with obesity.[7-9] The ratio of the oligomeric forms of adiponectin may affect insulin sensitivity, with higher concentrations of high-molecular-weight (HMW) adiponectin protecting against insulin resistance.[7,10] Circulating levels of leptin, which is also produced mainly by adipocytes and has pro-inflammatory properties, are positively correlated with amount of body fat.[7,10] In a recent case-control study including 73 MM patients and 73 controls,[11] MM was inversely associated with serum levels of adiponectin and was not associated with leptin. To our knowledge, these associations have not been investigated prospectively.

To determine whether pre-diagnostic circulating levels of leptin, total adiponectin, and HMW adiponectin are associated with future risk of MM, we conducted a nested case-control study in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial.

Methods

Methods for enrollment and specimen collection in PLCO have been described.[12] Briefly, between 1993 and 2001 approximately 155,000 individuals ages 55 to 74 years were enrolled in the study from ten U.S. cities. Screening-arm participants provided non-fasting blood samples that were processed and frozen within two hours of collection and stored at -70ºC. The trial was approved by institutional review boards at the National Cancer Institute and the ten study centers, and all participants provided written informed consent in accordance with the Declaration of Helsinki.

After excluding participants with a history of cancer (other than non-melanoma skin cancer) at baseline and those with a prior incident diagnosis of a lymphoid malignancy, we identified 174 patients with an incident diagnosis of MM (ICD-O-2-M 9732) on or before June 29, 2010 that had available pre-diagnostic heparin plasma (collected >1 year prior to diagnosis). Two controls were individually matched to each MM patient on age at baseline (5-year categories), sex, race, date of phlebotomy (3-month categories), time of day of phlebotomy (AM, PM) and study year of specimen collection.

Plasma concentrations of leptin, total adiponectin, and HMW adiponectin were measured in duplicate by enzyme-linked immunosorbent assay with reagents purchased from R&D Systems, Inc. (Minneapolis, MN). Samples from MM patients and their matched controls were analyzed consecutively in the same batch, and blinded quality control replicates were included in each batch. The overall coefficients of variation for total adiponectin, HMW adiponectin, and leptin were 2.7%, 4.7%, and 8.5%, respectively. All measurements were above the lower limits of detection of 3.9 ng/mL for total and HMW adiponectin and 15.6 pg/mL for leptin.

We used a t-test to assess differences in log-transformed levels of each analyte between MM patients and controls. Odds ratios (OR) and 95% confidence intervals (CI) were estimated using conditional logistic regression models with subjects assigned to quartiles of each analyte (averaged across duplicates) based on the distribution among controls. We further subdivided the highest quartile using the within-category median among controls to investigate associations across a wider range of exposure levels. Values for trend tests were assigned using the within-quartile medians. For analyses of each analyte as a continuous variable, we calculated ORs corresponding to a change in analyte levels of the interquartile range in controls. Analyses were performed with and without adjustment for body mass index (BMI). We also conducted analyses stratified below/above the median length of follow-up from blood collection to MM diagnosis (7.91 years) and by sex. Findings were considered statistically significant if two-sided P-values were <0.05.

Results and discussion

The distributions of matching factors among MM patients and controls were the same (Table 1). BMI, which is associated with MM in PLCO,[13] was slightly higher among selected MM patients than among controls. MM patients had lower levels of total and HMW adiponectin than did controls (P=0.001 and 0.003, respectively). BMI was positively correlated with leptin and negatively correlated with total and HMW adiponectin (Spearman correlation coefficients for leptin, total adiponectin, and HMW adiponectin were 0.52, -0.26, and -0.25, respectively, among controls and 0.59, -0.24, and -0.27 among MM patients).

As shown in Figure 1, we observed inverse associations between MM risk and plasma levels of total adiponectin (highest quartile vs. lowest: OR=0.49, 95% CI=0.26-0.93; P_{trend}=0.03) and HMW adiponectin (OR=0.44, 95% CI=0.23-0.85; P_{trend}=0.01). No consistent patterns of

association were observed for leptin. When the top quartile was subdivided using the within-category median, stronger associations were observed for those subjects with the highest levels of total adiponectin (OR=0.33, 95% CI=0.14-0.79) and HMW adiponectin (OR=0.32, 95% CI=0.14-0.75). These associations remained after restricting the analysis to MM patients diagnosed >7.91 years after blood collection and their matched controls (Table S1). Findings were similar among men and women (e.g., total adiponectin: $OR_{continuous}$=0.71 for men and 0.79 for women; Table S2). Risk estimates were essentially unchanged after adjustment for BMI, when leptin was included as a covariate in the analyses of total and HMW adiponectin, and after restricting to non-Hispanic whites (not shown).

We observed a modest association between BMI and MM risk in this analysis (OR per 5 kg/m^2 increase=1.14, 95% CI=0.94-1.39), the magnitude of which is consistent with the summary risk estimate from a meta-analysis of prospective studies of MM.[5] When we adjusted for adiponectin, which was negatively correlated with BMI, this association was attenuated by approximately 40% (ORs per 5 kg/m^2 increase of 1.08 and 1.09 after adjusting for HMW and total adiponectin, respectively).

Our study, to our knowledge the first prospective investigation of circulating adipokines and MM, shows a clear inverse relationship between total and HMW adiponectin levels and subsequent risk of MM, even among MM patients diagnosed approximately eight years or more after blood collection. These results suggest that adiponectin may play a role in the underlying biologic mechanisms linking obesity to myelomagenesis. Although the mechanisms are not fully understood, adiponectin may prevent MM development by suppressing production of pro-inflammatory cytokines such as IL-6 and TNF and inhibiting NF-kappa B activation while inducing other anti-inflammatory cytokines such as IL-10 and IL-1RA, thereby affecting

transduction pathways associated with survival and proliferation of malignant plasma cells.[10,14-16] Insulin and IGF-1 also promote myeloma cell growth and migration,[14,17-19] and it has been suggested that HMW adiponectin is critical in determining insulin sensitivity.[7,10] Our findings are particularly intriguing given that recent work has found host-derived adiponectin to be tumor-suppressive and a potential novel therapeutic target for MM and associated bone disease.[20] Confirmation of these findings in other prospective studies is needed.

Acknowledgements

Authorship

Contribution: JNH and MPP led the study design, statistical analysis, and preparation of the manuscript; LML, DB, GA, QL, and NR also contributed to the study design and analysis; YW conducted the assays in the laboratory of MNP; RMP advised and contributed to the statistical analysis; OL contributed to the analysis and interpretation of results; and all authors provided intellectual input into preparation of the manuscript.

Conflicts of interest: The authors declare no competing financial interests.

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References

1. American Cancer Society. Cancer Facts & Figures 2012. Atlanta, GA: American Cancer Society; 2012.

2. Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood* 2009;113(22):5412-7.

3. De Roos AJ, Baris D, Weiss NS, Herrinton LJ. Multiple myeloma. In: Schottenfeld D, Fraumeni JF, eds. Cancer Epidemiology and Prevention, 3rd Edition. New York, NY: Oxford University Press; 2006:919-45.

4. Alexander DD, Mink PJ, Adami HO, et al. Multiple myeloma: a review of the epidemiologic literature. *Int J Cancer* 2007;120 Suppl 12:40-61.

5. Wallin A, Larsson SC. Body mass index and risk of multiple myeloma: a meta-analysis of prospective studies. *Eur J Cancer* 2011;47(11):1606-15.

6. Landgren O, Rajkumar SV, Pfeiffer RM, et al. Obesity is associated with an increased risk of monoclonal gammopathy of undetermined significance among black and white women. *Blood* 2010;116(7):1056-9.

7. Roberts DL, Dive C, Renehan AG. Biological mechanisms linking obesity and cancer risk: new perspectives. *Annu Rev Med* 2010;61:301-16.

8. Greenberg AS, Obin MS. Obesity and the role of adipose tissue in inflammation and metabolism. *Am J Clin Nutr* 2006;83(2):461S-5S.

9. van Kruijsdijk RC, van der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. *Cancer Epidemiol Biomarkers Prev* 2009;18(10):2569-78.

10. Tilg H, Moschen AR. Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol* 2006;6(10):772-83.

11. Dalamaga M, Karmaniolas K, Panagiotou A, et al. Low circulating adiponectin and resistin, but not leptin, levels are associated with multiple myeloma risk: a case-control study. *Cancer Causes Control* 2009;20(2):193-9.

12.   Hayes RB, Reding D, Kopp W, et al. Etiologic and early marker studies in the prostate, lung, colorectal and ovarian (PLCO) cancer screening trial. *Control Clin Trials* 2000;21(6 Suppl):349S-55S.

13.   Troy JD, Hartge P, Weissfeld JL, et al. Associations between anthropometry, cigarette smoking, alcohol consumption, and non-Hodgkin lymphoma in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. *Am J Epidemiol* 2010;171(12):1270-81.

14.   Klein B, Tarte K, Jourdan M, et al. Survival and proliferation factors of normal and malignant plasma cells. *Int J Hematol* 2003;78(2):106-13.

15.   Wolf AM, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem Biophys Res Commun* 2004;323(2):630-5.

16.   Ajuwon KM, Spurlock ME. Adiponectin inhibits LPS-induced NF-kappaB activation and IL-6 production and increases PPARgamma2 expression in adipocytes. *American journal of physiology Regulatory, integrative and comparative physiology* 2005;288(5):R1220-5.

17.   Qiang YW, Yao L, Tosato G, Rudikoff S. Insulin-like growth factor I induces migration and invasion of human multiple myeloma cells. *Blood* 2004;103(1):301-8.

18.   Sprynski AC, Hose D, Caillot L, et al. The role of IGF-1 as a major growth factor for myeloma cell lines and the prognostic relevance of the expression of its receptor. *Blood* 2009;113(19):4614-26.

19.   Sprynski AC, Hose D, Kassambara A, et al. Insulin is a potent myeloma cell growth factor through insulin/IGF-1 hybrid receptor activation. *Leukemia* 2010;24(11):1940-50.

20.   Fowler JA, Lwin ST, Drake MT, et al. Host-derived adiponectin is tumor-suppressive and a novel therapeutic target for multiple myeloma and the associated bone disease. *Blood* 2011;118(22):5872-82.

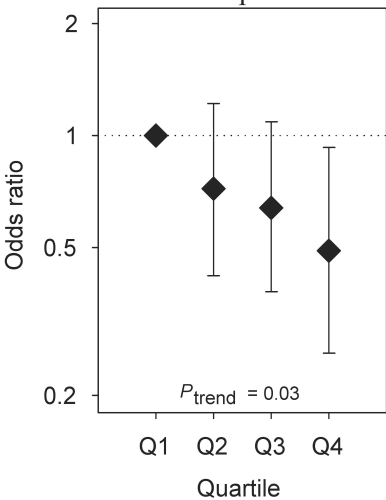**Table 1. Selected characteristics of multiple myeloma (MM) patients and matched controls**

Characteristic	MM patients (N=174)	Controls (N=348)
Age category, N (%)		
55-59 years	44 (25.3)	88 (25.3)
60-64 years	44 (25.3)	88 (25.3)
65-69 years	59 (33.9)	118 (33.9)
70-74 years	27 (15.5)	54 (15.5)
Sex, N (%)		
Female	62 (35.6)	124 (35.6)
Male	112 (64.4)	224 (64.4)
Race, N (%)		
White, non-Hispanic	158 (90.8)	316 (90.8)
Black, non-Hispanic	8 (4.6)	16 (4.6)
Hispanic	4 (2.3)	8 (2.3)
Asian/Pacific Islander	4 (2.3)	8 (2.3)
Body mass index, mean (SD)	27.7 (5.2)	27.2 (4.5)
Adiponectin (µg/mL), geometric mean (GSD)	8.72 (1.81)	9.60 (1.89)*
HMW adiponectin (µg/mL), geometric mean (GSD)	4.97 (2.07)	5.55 (2.18)*
Leptin (ng/mL), geometric mean (GSD)	10.01 (2.64)	9.60 (2.71)

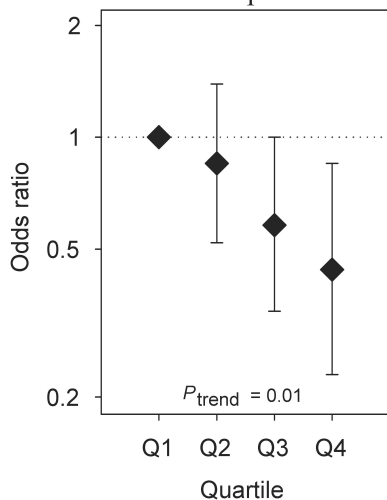Abbreviations: SD, standard deviation; GSD, geometric standard deviation; HMW, high molecular weight.
* $P=0.001$ and 0.003 for total and HMW adiponectin, respectively. To account for the matched design, we used a bootstrap procedure that resampled each case and the two matched controls as the independent unit and computed the t-test statistic for each bootstrap dataset for the log-transformed value of each analyte and for BMI. P-values are based on the bootstrap distribution function with 5000 bootstrap replications.

Figure 1. Risk of multiple myeloma in relation to pre-diagnostic circulating levels of total adiponectin, high molecular weight (HMW) adiponectin, and leptin in the PLCO Cancer Screening Trial