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Adiponectin and resistin are associated with risk for myelodysplastic syndrome, independently from the insulin-like growth factor-I (IGF-I) system

Maria Dalamaga[a], *Konstantinos Karmaniolas*[b], *Athina Nikolaidou*[a], *John Chamberland*[c], *Alex Hsi*[c], *Amalia Dionyssiou-Asteriou*[a], *Christos S. Mantzoros*[c,*]

[a]*Department of Clinical Biochemistry, Medical School, University of Athens, "Attikon" General University Hospital, 1 Rimini Street, Chaidari, 12462 Athens, Greece*

[b]*Department of Internal Medicine-Hematology Section, NIMTS General Hospital, 12 Monis Petraki and Vas.Sofias Avenue, 11528 Athens, Greece*

[c]*Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center, Boston, MA 02215, USA*

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ABSTRACT

Aim: Obesity has been implicated in the aetiology of myelogenous leukaemia and myelodysplasia (MDS). We hypothesised that altered secretion of adiponectin and resistin may underlie this association. We thus investigated the role of both total and high molecular weight (HMW) adiponectin and resistin in MDS.

Methods: In a case-control study, we studied 101 cases with incident, histologically confirmed primary MDS and 101 controls matched on gender and age between 2004 and 2007. Total and HMW adiponectin, resistin, insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein (IGFBP-3) were determined.

Results: Lower serum total or HMW adiponectin and/or resistin levels were independently associated with higher risk of MDS controlling for age, gender, BMI and serum levels of leptin, IGF-I and IGFBP-3 ($p < 0.002$). Although total and HMW adiponectin were both significantly inversely associated with MDS when modelled either in quartiles or continuously, HMW did not offer any substantial additional predictive value over total adiponectin (Odds ratio (OR) = 0.91 versus 0.93 for a 1 μg/ml change, respectively). IGF-I was positively associated with MDS by bivariate analysis and both IGF-I and IGFBP-3 were higher in advanced MDS and higher risk stages, but were not significantly and independently associated with MDS.

Conclusion: Total and HMW adiponectin may have a protective role in MDS, whereas resistin levels may be decreased via a compensatory mechanism.

1. Introduction

Myelodysplastic syndrome (MDS) defines a group of heterogeneous clonal haematopoietic stem cell disorders affecting mostly the elderly. MDS is characterised by trilineage defects in haematopoiesis leading to fatal cytopenias and to a variable risk of progression towards acute myeloid leukaemia (AML).[1–3] Although the precise incidence of primary MDS

* *Corresponding author*: Tel.: +1 617 667 8630; fax: +1 617 667 8634.
E-mail address: cmantzor@bidmc.harvard.edu (C.S. Mantzoros).

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has yet to be accurately assessed, its incidence is approximately twice that of AML amounting to 6–10 cases per 100,000 individuals.[4,5]

MDS generally arises *de novo* (idiopathic or primary MDS), but may also be seen after exposure to radiotherapy and/or cytotoxic chemotherapy – especially with alkylating agents and/or topoisomerase II inhibitors (secondary MDS).[2,5] However, until recently, the mechanisms underlying primary MDS have remained obscure. Senescence of the haematopoietic system, genetic disorders and occupational exposure to agents such as benzene have been suggested as predisposing risk factors.[2,5] Tobacco smoking[6–8] and alcohol consumption[8,9] have also been implicated as risk factors in several but not all studies,[2] whereas recent studies have indicated that obesity contributes to the aetiology of AML/MDS.[10–12]

A strong association of obesity with insulin resistance, characterised by hyperinsulinaemia, has been well documented[13] and there is evidence that insulin-like growth factors (IGFs) are implicated in several human malignancies related to obesity[14] including childhood leukaemia.[15] Adipose tissue is now widely considered an active endocrine organ producing several bioactive adipokines which regulate physiological and pathological processes, such as appetite, insulin sensitivity and resistance, inflammation, haematopoiesis, immunity and angiogenesis.[16,17] Adiponectin, an endogenous insulin sensitiser, has been found to exert a protective role for several types of malignancies *in vivo*, notably those related to obesity[18–23] including childhood myeloblastic leukaemia.[24] Moreover, adiponectin inhibits proliferation of myelomonocytic lineage cells and increases apoptosis *in vitro*.[25] However, it remains unknown whether high molecular weight (HMW) adiponectin, the presumably more active form of adiponectin, has a better predictive value than total adiponectin. Another hormone that has attracted significant attention recently is resistin, a novel hormone secreted by adipocytes and mononuclear cells. Resistin was initially discovered as a hormone related to insulin resistance, but more recent studies in mice and humans have revealed conflicting data indicating that the physiologic role of resistin may mainly be related to inflammation.[26] We thus attempted to explore whether these adipokines are associated with risk for MDS independently of the insulin-like growth factor-I (IGF-I) system, a hormonal system linked with several obesity and insulin resistance associated malignancies including leukaemia.[13–15]

Although we have recently studied total adiponectin,[27] no previous study has focused on IGF-I, its binding protein insulin-like growth factor binding protein (IGFBP-3), HMW adiponectin and resistin as prediction of MDS risk and no previous study has jointly evaluated all these hormones in relation to MDS risk. In this case-control study, we explore the role of total and HMW adiponectin and resistin levels in the aetiopathogenesis of MDS after adjusting for leptin and IGF-I, IGFBP-3, and evaluate their association with established prognostic factors.

2. Patients and methods

Cases and controls were recruited from patients hospitalised at the Veterans' Administration General Hospital of Athens (NIMTS). This hospital is the only Veteran's Hospital in Athens Metropolitan area and the entire Southern Greece. The study covered 101 cases and 101 controls under 83 years old from the same study base. Subjects were of Greek nationality and permanent residents of Greece. Medical records were reviewed and interviews were carried out to obtain information on demographic characteristics, medical history as well as weight and height.

2.1. Selection of cases

Eligible cases included newly diagnosed patients with histologically confirmed primary MDS, under age 83, consecutively admitted to the Internal Medicine Department – Haematology Section of the Veterans' Hospital between 20th February 2004 and 21st April 2007. A total of 110 cases were identified and of those 101 cases (59 males and 42 females), aged 58–83 years (median age: 70) consented to participate and were interviewed. Responders and non-responders did not differ on demographic variables, notably age, sex and time of diagnosis.

2.2. Selection of controls

Controls were patients, under age 83, admitted for non-neoplastic and non-infectious conditions to the Orthopedic and Opthalmologic Department of the same hospital and matched to cases on age (±5 years), gender and year/month of diagnosis (±1 month). No control developed MDS. The main causes of admission to the hospital in the control group were scheduled hip (30.7%) or knee joint replacement (17.8%) due to osteoarthritis, injuries, in particular fractures not secondary to a disease, (18.8%) and scheduled cataract operation (32.7%). For every eligible case, an attempt was made to randomly identify a control admitted to the Veterans' Administration General Hospital as closely as possible in time to the admission of the corresponding case (±1 month). A total of 118 potential controls were identified and of those 101 consented to participate and were interviewed. Amongst the latter, 59 were males and 42 females, aged 57–83 years (median age, 69). As for cases, the main reason for non-participation was refusal on the part of the subject or his/her relatives, but responders and non-responders did not differ on demographic variables, notably age, sex and time of diagnosis.

All cases and controls who participated in our investigation were fully informed of the aim of the study and gave written consent for their participation and their agreement that, provided that their anonymity is maintained, the results of this study may be presented or published, solely in the interest of science.

2.3. Diagnostic procedure, specimen collection and laboratory analysis

The main procedures and tests performed for MDS diagnosis were bone marrow aspirates, trephine biopsy, cytogenetics by means of karyotypic analysis using G-banding with trypsin-Giemsa stain (GTG) banding and peripheral blood count. All cases were classified according to the French–American–British (FAB) Cooperative Group scheme.[3] The criteria proposed by the FAB group for subclassification of these disorders are

morphological and comprise the percentage of myeloblasts and ring sideroblasts in the bone marrow, the percentage of blasts and monocytes in the peripheral blood and the presence of Auer rods. Based upon the above parameters, the following MDS subtypes were recognised: refractory anaemia (RA) (30.7%), refractory anaemia with ring sideroblasts (RARS) (21.8%), refractory anaemia with excess blasts (RAEB) (23.8%), refractory anaemia with excess blasts in transformation (RAEB-t) (9.9%) and chronic myelomonocytic leukaemia (CMML) (13.9%). The predominant histologic subtype of MDS in this series was RA (31 cases).

Based on the International Prognostic Scoring System (IPSS), MDS patients were also categorised as low risk MDS (47.5%) by virtue of their infrequent transformation to acute myelogenous leukaemia and good prognosis, intermediate-1 risk MDS (5%), intermediate-2 risk MDS (16.8%) and high risk MDS (30.7%). IPSS classifies MDS patients according to percentage of blasts in the bone marrow, number of cytopenias and cytogenetics.[28] Detailed description of subjects and methods has been recently described and results on leptin and adiponectin have recently been published.[27]

All blood specimens were collected prior to the initiation of chemotherapy or blood transfusions for the cases and prior to any therapeutic approach, including surgery, for the control group. Peripheral blood samples were centrifuged in the laboratory. Serum was separated and stored at –80 °C. Then all coded samples were shipped in one batch to the Beth Israel Deaconess Medical Center, in Boston, United States of America (USA). Adiponectin was determined by radioimmunoassay (LINCO Research, St. Charles, MO) with a sensitivity of 1 ng/ml, an intra-assay coefficient of variation of 1.8–6.2%, and an inter-assay coefficient of variation of 6.9–9.3%. Serum HMW adiponectin levels were measured using enzyme linked immuno sorbent assay (ELISA) (ALPCO Diagnostics, Salem, NH). The sensitivity of this assay was 0.04 ng/ml. The recovery rate was 99–103% for total adiponectin and 97–105% for HMW adiponectin. IGF-I and IGFBP-3 concentrations were measured using a commercially available immunoradiometric assay kit (DSL, Webster, TX). The sensitivity of the assay was 2 ng/ml. Serum resistin levels were determined by radioimmunoassay (LINCO Research Institute, St. Louis, MO) with a sensitivity of 0.78 ng/ml and an intra-assay coefficient of variation at 3.7 ng/ml being 3.6%.

2.4. *Statistical analysis*

Descriptive characteristics of MDS case and control subjects are presented as proportions or as mean values ± standard deviation (SD). Comparisons between cases and controls were conducted by using χ^2 tests for categorical variables and t-tests for continuous variables. One-way ANOVA with Bonferroni correction was conducted to compare cases amongst different subgroups. Log transformations were performed on hormonal variables prior to analysis to achieve normal distribution. Non-parametric Spearman correlation test was conducted to examine the associations of hormones and anthropometric characteristics amongst the controls. In the case-control analyses, samples were stratified by all subject based quartiles as well as control based quartiles of HMW adiponectin, resistin, IGF-I and IGFBP-3 to test the association of each hormone with MDS. Each model used simple and multivariate unconditional logistic regression models to produce crude and adjusted estimates. Hormones were also modelled as continuous variables in simple and multivariate logistic regression models. Statistical analysis of the data was performed with Statistical analytical system (SAS) 9.1 for Windows XP (SAS Institute, Cary, NC).

3. Results

Cases and controls had been matched by gender, age (within 5 years) and date of diagnosis (within 1 month). Cases had significantly higher height and weight than control subjects. IGF-I was found to be significantly higher in cases than in controls (Table 1), but cases had significantly lower serum levels of adiponectin, resistin and HMW than controls.

Table 2 shows the associations of hormones with MDS type, IPSS and karyotype amongst cases. IGFBP-3 levels are significantly different in MDS type and IPSS stratification schemes both before and after adjusting age, gender and body mass index (BMI). Amongst MDS subtypes, RARs, RAEB and RAEB-t have higher IGFBP-3 than RA and CMML ($p < 0.05$ in all comparisons). In IPSS, subjects in the high risk subgroup had significantly higher IGFBP-3 level than those in the low risk subgroups ($p < 0.008$). IGF-I levels were found to be significantly different amongst MDS subtypes with RAR and RAEB-t having strong significantly higher IGF-I serum level than RA and CMML ($p < 0.01$). MDS types of HMW levels are found to have a borderline significance, but the significance was removed after adjusting age, gender and BMI. Significant differences of IGFBP-3 levels amongst karyotype classes became non-significant after the adjustment.

The unadjusted and BMI adjusted associations between hormone levels and anthropometric characteristics from controls and cases are presented in Table 3. Amongst controls, HMW is significantly and positively associated with total adiponectin (Table 3a). IGF-I and IGFBP-3 are significantly associated with each other. Amongst cases, IGF-I was found to be significantly and negatively associated with adiponectin and HMW adiponectin, and IGFBP-3 was significantly and negatively associated with HMW adiponectin (Table 3c). The direction and magnitude of associations were not altered after adjusting for BMI (Tables 3b and 3d).

Table 4 displays the odd ratios for MDS in relation to HMW adiponectin, resistin, IGF-I and IGFBP-3 levels. In unadjusted analyses, subjects in the highest and second highest quartiles of HMW adiponectin have significantly lower risk of MDS than subjects in the lowest quartile (Q3 versus Q1: p-value = 0.008; Q4 versus Q1: p-value = 0.002). However, the respective p-values become non-significant after adiponectin is added to the models (Table 4a). Subjects in all other quartiles of resistin levels have significantly lower odds of developing MDS in comparison to the referent quartile (Table 4b). Importantly, adjustments for potential confounding factors do not alter the associations between resistin and MDS (Table 4b). In the unadjusted model of IGF-I, subjects in the highest quartile demonstrated a trend towards lower risk of MDS than subjects in the lowest quartile, but these associations are not particularly strong (Odds ration (OR) = 2.30, 95%; confidence

Table 1 – Descriptive characteristics of myelodysplasia (MDS) cases subjects (n = 101) and controls subjects (n = 101)

	Cases	Controls	*p*-Value
n	101	101	
Male, *n*/female, *n*	59/42	59/42	1.0
Age, years, mean (standard deviation (SD))	69.3 (5.6)	69.1 (5.6)	0.80
Weight, kg,, mean (SD)	77.0 (8.9)	72.4 (8.7)	<0.001
Height, m, mean (SD)	1.71 (0.07)	1.67 (0.07)	<0.001
Body mass index (BMI), mean (SD)	26.4 (2.4)	25.9 (2.1)	0.12
Resistin, ng/ml, mean (SD)	20.6 (30.6)	28.8 (17.4)	0.019
Adiponectin, μg/ml, mean (SD)	11.8 (7.8)	16.3 (8.0)	<0.001
High molecular weight (HMW), μg/ml, mean (SD)	5.3 (4.6)	7.5 (5.6)	0.003
Insulin-like growth factor-I (IGF-I), ng/ml, mean (SD)	322.9 (200.1)	260.4 (169.4)	0.018
Insulin-like growth factor binding protein (IGFBP-3), ng/ml, mean (SD)	3472.5 (1392.6)	3357.5 (1223.4)	0.534
MDS, *n* (%)			
Refractory anaemia (RA)	31 (30.7)		
Refractory anaemia with ring sideroblasts (RARS)	22 (21.8)		
Chronic myelomonocytic leukaemia (CMML)	14 (13.9)		
Refractory anaemia with excess blasts (RAEB)	24 (23.7)		
Refractory anaemia with excess blasts in transformation (RAEB-t)	10 (9.9)		
International Prognostic Scoring System (IPSS), *n* (%)			
Low risk	48 (47.5)		
Intermediate-1 risk	5 (5.0)		
Intermediate-2 risk	17 (16.8)		
High risk	31 (30.7)		
Karyotype, *n* (%)			
Normal	43 (42.6)		
Good prognosis	17 (16.8)		
Miscellaneous	14 (13.9)		
Poor prognosis	27 (26.7)		

interval (CI) (1.06–5.00)). Adjusting for covariates rendered these associations non-significant (Table 4c). Finally, no significant associations between MDS and IGFBP-3 were detected (Table 4d). Similar to previously reported data,[27] low adiponectin levels are a significant predictor of MDS. Total adiponectin does not offer any substantially better predictive value than HMW adiponectin.

Continuous associations between HMW and total adiponectin with MDS were also tested (data not shown). After adjustment for age, gender, height, weight, BMI, resistin, leptin, IGF-I, and IGFBP-3, the OR for a 1 μg/ml increase in HMW adiponectin was 0.91 (95% CI (0.85–0.98), $p = 0.01$) and the OR for a 1 μg/ml increase in total adiponectin was 0.93 (95% CI (0.89–0.98), $p = 0.002$). Continuous total and HMW adiponectin both remained significantly inversely associated with MDS after adjustment for all covariates. The statistical significance of the association was attenuated for both total and HMW adiponectin in Model 4, however, after mutual adjustment and became non-significant. Resistin was not significantly associated with MDS after adjustment for age, gender, height, and weight when modelled continuously ($p = 0.12$). As seen with quartiles, IGF-I and IGFBP-3 were not significantly associated with MDS in continuous models.

4. Discussion

The results of this case-control study demonstrate that both higher serum total and HMW adiponectin levels are associated with lower risk of MDS before and after controlling for age, gender, BMI as well as serum levels of resistin, leptin, IGF-I and IGFBP-3. These findings are in accordance with *in vitro* data demonstrating that the adipocyte secreted hormone adiponectin induces apoptosis through down-regulation of bcl-2 expression and suppresses proliferation of myeloid cell lineage.[25] The findings reported herein are also consistent with similar findings from our prior case-control study reporting low total adiponectin levels as a risk factor for childhood myeloblastic leukaemia.[24]

Several epidemiologic studies have shown inverse associations between adiponectin levels and risk for breast,[18] endometrial,[19] prostate,[20] gastric,[21] renal,[22] colorectal,[23] cancers as well as childhood AML[24] and melanoma.[29] Adiponectin circulates in trimer, hexamer and HMW multimers; the latter has been proposed to represent the most bioactive form of adiponectin.[30] When compared with total adiponectin, HMW adiponectin has been suggested to be a better predictor of insulin sensitivity and metabolic parameters.[31] We explored for the first time the predictive value of HMW adiponectin and found that HMW is significantly and inversely associated with MDS risk. HMW adiponectin does not provide any additional information over determinations of total adiponectin, however. Hence, both low serum HMW and total adiponectin levels are associated with high MDS risk independently of other serum hormones or other known risk factors.

In this study, subjects with MDS were taller and heavier than controls whilst differences of BMI were only of borderline significance. Several recent studies reported that obesity is significantly associated with risk for AML and leukaemia[10–12,32] in

Table 2 – Resistin, HMW adiponectin, IGF-I and IGFBP-3 levels in subgroups of MDS subjects subdivided as per different classifications

	HMW (μg/ml)			Resistin (ng/ml)			IGF-I (ng/ml)			IGFBP-3 (ng/ml)		
	Mean (standard error (SE))		*p*-Value	Mean (SE)		*p*-Value	Mean (SE)		*p*-Value	Mean (SE)		*p*-Value
(*a*) *Unadjusted*												
MDS			0.028			0.658			<0.001			<0.001
RA	7.23	(0.80)		24.8	(5.5)		228.5	(32.1)		2631.5	(217.1)	
RARS	3.84	(0.94)		24.4	(6.6)		452.0	(38.2)		4083.6	(257.7)	
CMML	5.86	(1.18)		12.1	(8.2)		222.6	(47.8)		2733.3	(323.1)	
RAEB	4.70	(0.90)		16.3	(6.3)		335.3	(36.5)		4210.0	(246.8)	
RAEB-t	3.13	(1.40)		21.3	(9.8)		442.2	(56.6)		3999.8	(382.3)	
IPSS			0.153			0.333			0.217			0.026
Low risk	6.16	(0.66)		25.2	(4.4)		304.6	(28.7)		3163.2	(195.6)	
Intermediate risk	5.04	(0.97)		17.9	(6.5)		290.0	(42.4)		3380.8	(288.9)	
High risk	4.14	(0.82)		15.2	(5.5)		374.7	(35.7)		4016.4	(243.4)	
Karyotype			0.667			0.297			0.666			0.031
Normal	5.78	(0.70)		23.3	(4.7)		309.8	(30.7)		3020.6	(206.1)	
Good prognosis	5.38	(1.12)		28.8	(7.4)		334.3	(48.9)		3998.4	(327.7)	
Miscellaneous prognosis	4.02	(1.24)		9.9	(8.2)		282.8	(53.9)		3552.2	(361.1)	
Poor prognosis	5.14	(0.89)		16.6	(5.9)		357.3	(38.8)		3819.6	(260.0)	
(*b*) *After adjustment for age, sex, and BMI*												
MDS			0.065			0.535			<0.001			<0.001
RA	7.0	(0.78)		25.5	(5.4)		231.3	(33.1)		2675.3	(214.0)	
RARS	3.72	(0.91)		24.1	(6.3)		452.2	(38.8)		4067.0	(250.4)	
CMML	5.71	(1.14)		12.2	(7.9)		225.0	(48.7)		2777.1	(314.3)	
RAEB	4.93	(0.89)		14.9	(6.2)		332.2	(37.9)		4157.6	(244.8)	
RAEB-t	3.78	(1.37)		22.8	(9.5)		437.0	(58.4)		3964.7	(376.8)	
IPSS			0.332			0.268			0.269			0.05
Low risk	5.90	(0.64)		25.7	(4.3)		310.8	(29.4)		3229.7	(191.2)	
Intermediate risk	5.30	(0.94)		17.1	(6.3)		283.8	(43.1)		3316.4	(280.3)	
High risk	4.37	(0.79)		15.1	(5.3)		369.4	(36.3)		3959.0	(235.9)	
Karyotype			0.641			0.230			0.777			0.16
Normal	5.26	(0.70)		23.3	(4.6)		320.4	(32.2)		3099.7	(208.8)	
Good prognosis	6.32	(1.14)		28.3	(7.6)		312.8	(52.9)		3818.0	(342.4)	
Miscellaneous prognosis	4.18	(1.18)		7.6	(7.9)		285.3	(54.6)		3650.7	(353.6)	
Poor prognosis	5.30	(0.85)		18.1	(5.7)		352.8	(39.4)		3756.2	(255.0)	

Table 3a – Spearman correlation coefficients of study variables amongst controls

	Weight	Height	BMI	Leptin	Resistin	Adiponectin	HMW	IGF-I	IGFBP-3
Age	–0.066	–0.112	0.026	–0.045	–0.043	–0.078	–0.063	–0.102	–0.165
Weight		0.767***	0.772***	0.003	–0.02	0.053	0.029	0.171	0.103
Height			0.219*	0.053	0.018	0.107	0.140	0.100	0.015
BMI				–0.095	–0.050	–0.011	–0.094	0.169	0.15
Leptin					0.060	–0.263**	–0.229*	–0.016	0.134
Resistin						–0.07	–0.057	0.015	–0.143
Adiponectin							0.885***	–0.016	–0.115
HMW								0.036	–0.139
IGF-I									0.647***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3b – Spearman correlation coefficients of study variables amongst controls after adjusting for BMI

	Weight	Height	Leptin	Resistin	Adiponectin	HMW	IGF-I	IGFBP-3
Age	–0.134	–0.120	–0.043	–0.041	–0.078	–0.061	–0.108	–0.171
Weight		0.964***	0.120	0.029	0.096	0.167	0.064	–0.021
Height			0.076	0.029	0.112	0.168	0.065	–0.018
Leptin				0.056	–0.265**	–0.240*	–0.00002	0.150
Resistin					–0.071	–0.061	0.024	–0.137
Adiponectin						0.887***	–0.014	–0.115
HMW							0.054	–0.126
IGF-I								0.638***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3c – Spearman correlation coefficients of study variables amongst cases

	Weight	Height	BMI	Leptin	Resistin	Adiponectin	HMW	IGF-I	IGFBP-3
Age	–0.105	–0.008	–0.114	–0.108	0.086	0.168	0.223*	–0.066	–0.114
Weight		0.585***	0.673***	0.033	0.048	–0.117	–0.065	–0.020	–0.106
Height			–0.158	0.152	–0.034	–0.216*	–0.221*	–0.0008	–0.065
BMI				–0.067	0.071	0.001	0.069	0.012	–0.060
Leptin					–0.335***	–0.262**	–0.267**	0.412***	0.638***
Resistin						0.178	0.143	0.066	–0.008
Adiponectin							0.927***	–0.386***	–0.173
HMW								–0.418***	–0.217*
IGF-I									0.671***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 3d – Spearman correlation coefficients of study variables amongst cases after adjusting for BMI

	Weight	Height	Leptin	Resistin	Adiponectin	HMW	IGF-I	IGFBP-3
Age	–0.038	–0.010	–0.117	0.095	0.169	0.233*	–0.065	–0.122
Weight		0.946***	0.106	0.0001	–0.159	–0.151	–0.037	–0.089
Height			0.143	–0.023	–0.218*	–0.213*	0.003	–0.076
Leptin				–0.332***	–0.262**	–0.263**	0.414***	0.637***
Resistin					0.179	0.139	0.065	–0.003
Adiponectin						0.929***	–0.386***	–0.173
HMW							–0.420***	–0.214*
IGF-I								0.673***

*$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

general as well as MDS.[27] Potential mechanisms that link obesity to MDS and AML include an augmented total number of haematopoietic stem cells that could undergo malignant transformation. Several endocrine factors could play an important role in this regard, including the endogenously secreted insulin sensitiser adiponectin which may have a protective

Table 4a – Odd ratios and 95% confidence intervals for risk of MDS in relation to HMW adiponectin by control-defined quartiles

	Quartile of HMW				*p*-Value
	Q1	Q2	Q3	Q4	
Cases (*n*)/Total (*N*) (%)	47/72 (65.3)	25/50 (50)	16/41 (39.0)	13/38 (34.2)	
HMW range, μg/ml	0.125–3.634	3.669–5.968	6.246–9.175	9.216 – 31.099	
Cases versus control subjects					
Crude	1.0	0.53 (0.26–1.11)	0.34 (0.15–0.75)	0.28 (0.12–0.63)	0.007
Model 1	1.0	0.55 (0.25–1.23)	0.41 (0.18–0.96)	0.27 (0.11–0.67)	0.0264
Model 2	1.0	0.55 (0.24–1.24)	0.44 (0.19–1.04)	0.30 (0.12–0.74)	0.0495
Model 3	1.0	0.56 (0.23–1.33)	0.36 (0.14–0.90)	0.29 (0.11–0.78)	0.0485
Model 4	1.0	0.76 (0.29–1.98)	0.69 (0.21–2.29)	0.98 (0.17–5.84)	0.811

Model 1: age, gender, height, weight, adjusted.
Model 2: age, gender, height weight and BMI adjusted.
Model 3: age, gender, height, weight, BMI, resistin, leptin, IGF-I and IGFBP-3 adjusted.
Model 4: age, gender, height, weight, BMI, resistin, leptin, IGF-I, IGFBP-3 and adiponectin adjusted.

Table 4b – Odd ratios and 95% confidence intervals for risk of MDS in relation to resistin by control-defined quartiles

	Quartile of Resistin				*p*-Value
	Q1	Q2	Q3	Q4	
Cases (*n*)/Total (*N*) (%)	68/93 (73.1)	11/36 (30.6)	9/34 (26.5)	13/39 (33.3)	
Resistin range, ng/ml	0.505–17.347	17.347–26.825	26.825–35.834	35.834–185.518	
Cases versus control subjects					
Crude	1.0	0.16 (0.07–0.38)	0.13 (0.05–0.32)	0.18 (0.08–0.41)	<0.001
Model 1	1.0	0.19 (0.08–0.47)	0.15 (0.06–0.40)	0.21 (0.09–0.51)	<0.001
Model 2	1.0	0.18 (0.07–0.45)	0.16 (0.06–0.43)	0.23 (0.09–0.55)	<0.001
Model 3	1.0	0.19 (0.07–0.50)	0.12 (0.04–0.34)	0.20 (0.08–0.52)	<0.001
Model 4	1.0	0.17 (0.07–0.47)	0.11 (0.04–0.31)	0.20 (0.07–0.51)	<0.001

Model 1: age, gender, height, weight, adjusted.
Model 2: age, gender, height weight and BMI adjusted.
Model 3: age, gender, height, weight, BMI, HMW, leptin and adiponectin adjusted.
Model 4: age, gender, height, weight, BMI, HMW, leptin, adiponectin, IGF-I and IGFBP-3 adjusted.

Table 4c – Odd ratios and 95% confidence intervals for risk of MDS in relation to IGF-I by control-defined quartiles

	Quartile of IGF-I				*p*-Value
	Q1	Q2	Q3	Q4	
Cases (*n*)/Total (*N*) (%)	18/43	18/43	22/47	43/69	
IGF-I range, ng/ml	17.3–152.2	152.2–225.4	225.4–298.1	298.1–921.7	
Cases versus control subjects					
Crude	1.0	1.0 (0.43–2.36)	1.22 (0.53–2.81)	2.30 (1.06–5.00)	0.09
Model 1	1.0	0.74 (0.29–1.91)	1.15 (0.47–2.83)	1.71 (0.73–3.96)	0.26
Model 2	1.0	0.81 (0.31–2.11)	1.12 (0.45–2.81)	1.69 (0.71–3.98)	0.36
Model 3	1.0	0.97 (0.35–2.71)	1.41 (0.52–3.84)	1.80 (0.69–4.70)	0.49
Model 4	1.0	1.02 (0.33–3.17)	1.49 (0.48–4.67)	1.96 (0.56–6.81)	0.56

Model 1: age, gender, height, weight, adjusted.
Model 2: age, gender, height, weight and BMI adjusted.
Model 3: age, gender, height, weight, BMI, resistin, leptin and adiponectin adjusted.
Model 4: age, gender, height, weight, BMI, resistin, leptin, adiponectin, HMW and IGFBP-3 adjusted.

effect[24] as well as the IGF-I system which may have mitogenic and antiapoptotic properties,[33] and may be related to insulin resistance in addition to also promoting height.

Another mechanism that could serve as a link between obesity and MDS could be an impaired immune function associated with obesity,[16] which in turn, may be the result of decreased adiponectin and/or altered resistin.[26] Recent studies have shown interleukin-6 (IL-6) and tumour necrosis factor-α (TNF-α) over-expression in progenitor cells[34] in the bone marrow microenvironment of patients with MDS. Sub-

Table 4d – Odd ratios and 95% confidence intervals for risk of MDS in relation to IGFBP-3 by control-defined quartiles

	Quartile of IGFBP-3				*p*-Value
	Q1	Q2	Q3	Q4	
Cases (*n*)/Total (*N*) (%)	25/50	23/49	21/46	32/57	
IGFBP-3 range, ng/ml	524.6–2491	2491–3391.9	3391.9–4325.4	4325.4–6362.7	
Cases versus control subjects					
Crude	1.0	0.89 (0.40–1.95)	0.84 (0.38–1.87)	1.28 (0.60–2.74)	0.71
Model 1	1.0	0.75 (0.32–1.74)	0.68 (0.28–1.64)	1.00 (0.43–2.32)	0.74
Model 2	1.0	0.73 (0.31–1.72)	0.72 (0.29–1.77)	0.96 (0.40–2.27)	0.82
Model 3	1.0	0.93 (0.37–2.36)	0.98 (0.35–2.79)	1.18 (0.44–3.18)	0.96
Model 4	1.0	0.82 (0.32–2.13)	0.73 (0.24–2.24)	0.72 (0.22–2.35)	0.95

Model 1: age, gender, height, weight, adjusted.
Model 2: age, gender, height, weight and BMI adjusted.
Model 3: age, gender, height, weight, BMI, resistin, leptin and adiponectin adjusted.
Model 4: age, gender, height, weight, BMI, resistin, leptin, adiponectin, HMW and IGF-I adjusted.

Table 4e – Odd ratios and 95% confidence intervals for risk of MDS in relation to adiponectin by control-defined quartiles

	Quartile of adiponectin				*p*-Value
	Q1	Q2	Q3	Q4	
Cases (*n*)/Total (*N*) (%)	50/75 (66.7)	19/44 (43.2)	19/45 (42.2)	13/38 (34.2)	
Adiponectin range, μg/ml	0.268–10.158	10.275–14.361	14.378–21.092	21.264–42.543	
Cases versus control subjects					
Crude	1.0	0.38 (0.18–0.82)	0.37 (0.17–0.78)	0.26 (0.11–0.59)	0.004
Model 1	1.0	0.45 (0.20–1.03)	0.40 (0.18–0.90)	0.33 (0.14–0.79)	0.03
Model 2	1.0	0.44 (0.19–1.01)	0.44 (0.19–1.02)	0.37 (0.15–0.91)	0.07
Model 3	1.0	0.44 (0.18–1.07)	0.43 (0.18–1.02)	0.35 (0.13–0.91)	0.09
Model 4	1.0	0.56 (0.22–1.41)	0.70 (0.25–2.01)	1.05 (0.22–4.98)	0.52

Model 1: age, gender, height, weight, adjusted.
Model 2: age, gender, height, weight and BMI adjusted.
Model 3: age, gender, height, weight, BMI, resistin, leptin, IGF-I and IGFBP-3 adjusted.
Model 4: age, gender, height, weight, BMI, resistin, leptin, IGF-I, IGFBP-3 and HMW adiponectin adjusted.

sequently, reduced total and HMW adiponectin levels observed in myelodysplasia could be linked with the TNF-α overproduction in the bone marrow through a mechanism involving altered TNF-α transcription or translation.[34] Increased TNF-α levels are implicated in excessive intramedullary progenitor cell apoptosis and enhanced bone marrow angiogenesis, both of which are considered as main cofactors of inefficient haematopoiesis in MDS and progression towards AML.[34] Moreover, the anti-inflammatory activities of adiponectin comprise inhibition of IL-6 production in part through nuclear factor kB (NF-kB) suppression.[35] The enhanced NF-kB activity has been mainly attributed to the HMW adiponectin isoforms but these results need to be replicated.[36]

Resistin, an adipokine belonging to the family of resistin-like molecules (RELMs) was originally discovered as a molecule that induced insulin resistance or impaired hepatic sensitivity to insulin and caused hyperglycemia without affecting peripheral insulin sensitivity.[37] However, data in humans are controversial. In contrast to mice, resistin in humans is expressed in lower levels in adipocytes but at relatively higher levels in circulating blood monocytes.[38] Moreover, studies in humans have failed to detect higher serum resistin levels in obese or insulin resistant subjects.[26] More recently, resistin is seen mainly as an inflammatory factor which is associated with TNF-α and IL-6, and may upregulate several adhesion molecules and cytokines.[38] In this study, MDS patients have lower resistin levels, probably due to a compensatory response to the upregulation of other inflammatory factors aetiologically linked to myelodysplasia.

Since IGF-I mediates the effect of growth hormone and has been related to obesity associated cancers[14] including leukaemia[15] we explored the role of this hormone together with its principal binding protein IGFBP-3 in the aetiopathogenesis of MDS. Although IGF-I levels were significantly higher in MDS patients than controls in univariate analysis, there was no significant association between IGF-I or IGFBP-3 and the likelihood of MDS after adjustment with the other anthropometric and hormonal variables. This finding is consistent with a similar study reporting IGF-I and IGFBP-3 levels in patients with childhood leukaemia indicating the importance of IGF-I bioavailability.[15] Although not considered as a classical haematopoietic growth factor, IGF-I and its binding proteins exert diverse biological effects stimulating both erythroid and myeloid progenitor cells.[39] IGF-I produced by bone marrow stromal cells plays a pivotal role in regulating erythropoiesis, haeme synthesis, granulopoiesis and B-lymphopoiesis acting via the IGF-I receptor (IGF-IR).[39] IGF-IR over-expression was found in advanced stages of MDS and predicted malignant proliferation of haematopoietic cells leading to AML transfor-

mation.[40] Sensitivity of stem cells to IGF-I might also play an important role to leukaemogenesis. Hypersensitivity to IGF-I has been implicated in the aetiology of polycythemia vera, a myeloproliferative disorder.[39] In our study, we have found higher IGFBP-3 levels in higher risk MDS patients and higher IGF-I and IGFBP-3 levels in certain MDS subgroups, observations that are consistent with the above basic research findings and need to be replicated since they warrant further investigation. Amongst cases, adiponectin and HMW adiponectin were inversely and significantly associated with IGF-I, possibly reflecting an underlying insulin resistance. No such associations were observed amongst controls in accordance with a previous study reporting only modest associations of adiponectin with the IGF system, mainly mediated by body fat.[41]

This is the first study exploring separately and jointly the role of total and HMW adiponectin, resistin, leptin, IGF-I and IGFBP-3 in myelodysplasia. Cohort studies are generally considered as being superior to case-control investigations, but are extremely difficult to undertake in MDS due to the rarity of the disease. Thus, a case-control design is appropriate herein. We included hospital controls which were carefully matched to cases, and with admission diagnoses that are not known to be related with the principal exposure variables, namely hormone levels. Despite the rarity of MDS in the general population, we implemented an appropriately powered study, which, albeit of modest size, was sufficiently large to generate statistically significant associations with the above variables.

In conclusion, we have found biologically plausible and empirically strong evidence that total and HMW adiponectin may have a protective role in MDS. Moreover, we have found that HMW adiponectin does not offer any additional predictive value in comparison with total adiponectin. The possible association of resistin with MDS risk appears to be part of a compensatory response in this inflammatory state and needs to be explored further. Our data need to be replicated in other populations and the mechanisms underlying the role of adiponectin in myelopoiesis and leukaemogenesis require further investigation.

Conflict of interest statement

None declared.

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