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ASSOCIATION OF RETINOL BINDING PROTEIN4 (RBP4) AND GHRELINPLASMA LEVELSWITHINSULIN RESISTANCE AND DISEASE SEVERITY IN NON-DIABETIC PATIENTS WITH HIDRADENITIS SUPPURATIVA

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ABSTRACT

Hidradenitis suppurativa (HS) is a chronic inflammatory disease associated with insulin resistance (IR). Retinol binding protein 4 (RBP4) and ghrelin are two bioactive proteins that have been involved in glucose metabolism and IR, but also in the regulation of immune and inflammatory processes.The aim of this study was to determine the serum levels of RBP4 and ghrelin in patients with HS, and toassessthe possiblerelationship between these levels andIR, disease severity and HS risk. A total of 137 subjects (77 HS-patients and 60 controls) without diabetes mellitus were enrolled in thiscross-sectional study.Patients with HS had significantly higher RBP4 but lower ghrelin plasma levels than controls, independently of body mass index (BMI). Serum RBP4 levels were positively correlatedto disease severity and IR in HS patients. However, we found no association between ghrelin levels and any clinical or laboratory parameters. Moreover, high serum RBP4and low ghrelin levels were associated with an increased risk for HS. Our results suggest that high RBP4 levels may be a surrogate biomarker for IR in patients with HS. Moreover,increased RBP4 and decreased ghrelin levels could also be independent risk factors for the development of HS.

Keywords: Hidradenitis suppurativa; Retinol-binding protein 4; Ghrelin; Insulin resistance.

1 BACKGROUND

Hidradenitis suppurativa (HS) is a chronic, inflammatory, skin disease of the hair follicle. The pathogenesis of HS remains largely uncertain, although current evidence suggeststhe existence of an early phase of immune activation with progression to chronic inflammation.[1]In this sense, several pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-10 and IL-17 are considered to play a pivotal role in the HS pathogenic process.[1,2] Besides,someadipokines which can modulatepro-inflammatory cytokines levels, havealso been recently related to HS pathogenesis.[3,4]

HS patients exhibitan increased prevalence of subclinical atherosclerosis andhigh risk for majorcardiovascular (CV) events and CV mortality.[5-8] Furthermore, an elevated prevalence of metabolic disorders, including metabolic syndrome (MS),[9]insulin resistance (IR)[10]and type 2 diabetes mellitus (T2DM)[11]has also been reported in these patients. IR, a crucial pathophysiological factor for the development of MS and T2DM, and accelerated atherogenesis has been related to the chronic systemic inflammation state found inHS.[6,10] In this regard, it has been suggested thatraisedblood levels of certain pro-inflammatory cytokines involved in HS pathogenesis, such as IL-1β and IL-6,may also induce a pro-atherogenic and IR-adipokine pattern in HS patients.[1]

Plasma retinol-binding protein 4 (RBP4) is a 21-kDa protein that belongs to the lipocalin family and is the specific carrier for retinol in the blood circulation. It delivers vitamin A from the liver stores to the peripheral tissues.[12]Moreover, RBP4 alsoacts as a pro-inflammatory adipokine and has been recognized as a biomarker of IR in patients with obesity and T2DM, since it can impair insulin signaling.[12-14]Besides, this adipocyte-secreted hormone has also been involved in the atherosclerotic process and CV disease.[12]Thus, it has been suggested that inflammation may be the crucial pathway through which RBP4 might exert its role in the pathogenesis of IR and CV disease.[12,15]

Ghrelin, a peptide predominantly secreted by the stomach, is another emerging IR-related biomarker which also plays a role in modulating immune responses and inflammatory processes.[16] The secretion of both molecules,RBP4 and ghrelin,are dysregulated in several chronic inflammatory conditions.[17,18]

2 QUESTIONS ADDRESSED

We investigated whether there are differences in serum RBP4 and ghrelin levels in HS-patients compared to healthy controls. Furthermore, we sought to assess whether there is any relationship between these levels and IR, disease severity and HS risk.

3 STUDY DESIGN

3.1 Participants and protocol

Cross-sectional study including 137 participants (77 HS patients and 60 controls) recruited from our Dermatology outpatient clinic at the University Hospital Marques de Valdecilla (Santander, Northern Spain). Patients were ≥18 years and fulfilled the diagnostic criteria for HS.[19] The control group was set up with hospital medical staff and subjects who had been admitted at the Dermatology Department because of non-inflammatory disorders.The research protocol was approved by the local ethics committee, and all the participants gave written informed consent.

Exclusion criteria, clinical evaluation, and laboratory studies have been previously described.[3,10] Briefly, patients or controls with a history of CV events, DM and other endocrine diseases, chronic renal or liver failure, and/or other inflammatory cutaneous or systemic diseases, or taking drugs (in the previous 6 months) affecting carbohydrate metabolism, were excluded from the study. The severity of HS was assessed by the HS Physician Global Assessment (HS-PGA); HS was classified as moderate-severe-very severe (PGA ≥3) and as minimal-mild HS (PGA<3).

All the participants provided information on demographic features and past medical history. Body height and weight, body mass index (BMI), waist circumference (WC), systolic blood pressure (BP) and diastolic BP were measured in all patients and controls. Body mass index (BMI) was calculated as weight (kg)/ [height (m)]^2.

Blood samples were collected after overnight fasting. Glucose and insulin levels, glycated hemoglobin (HbA1c), triglycerides, serum total cholesterol (TC), high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c) and high-sensitivity C-reactive protein (hs-CRP) were assessed in all participants.MS was diagnosed by the presence of three or more criteria according to the National Cholesterol Education Program´s Adult Treatment Panel III (ATP III).[20]The degree of IR was calculated using the homeostatic model assessment for IR (HOMA-IR) expressed as fasting insulin level (μIU/ml) x fasting glucose level (mg/dl)/405. IR was diagnosedifHOMA-IR >2.5.[10] Serum levels of RBP4 and ghrelin were analyzed byenzyme-linked immunosorbent assay (Sigma-Aldrich Co. LLC, St. Louis, MO, USA). RBP4 andghrelin levels were expressed as μg/ml and as ng/l, respectively. Intra-and inter-assay coefficients of variationwere less than 10% for both analytes.

3.2 Statistical analysis

Results were expressed as numbers (percentage), mean±standard deviation (SD) or median and interquartile range (IQR), as appropriate.Mann-Whitney U-test and Chi-squared or Fisher testswere used to compare quantitative and qualitative variables respectively. To evaluate the relationship between serum RBP-4 and ghrelin levels and HOMA-IR, Pearson's correlation was used. Moreover, forward stepwise multivariable logistic regression models were built to assess the potential association between both proteins and IR, HS risk and HS severity.

4 RESULTS

4.1 Baseline features and serum RBP4 and ghrelin levels

Demographic, clinical and laboratory data of HS-patients and controls are summarized in Table I. There were no significant differences regarding age, sex, HbA1c, LDL-c and triglyceride levels. Compared with the control group, patients with HS displayed significantly higher values of BMI, WC, BP, hs-CRP, fasting glucose, fasting insulin, HOMA-IR index and prevalence of IR and MS.Moreover, HS-patients had lower serum HDL-c levels than the control group. RBP4 was significantly higher in HS-patients(59.3 μg/ml[47.2-72.7] vs. 38.5μg/ml[34.7-44.3]; p<0.0001) whilst serum ghrelin levels were significantly decreased in HS-patients compared to controls (50.0 ng/ml [50.0-186.3] vs. 210.5 ng/ml [117.7-302.9]; p<0.0001). These differences in RBP4 and ghrelin concentrations remained significant after adjusting by age, sex and BMI.

4.2 Association of RBP4 and ghrelin with IR and severity of HS

In patients with HS, we found a significantly positive correlation between RBP4 levels and IR (r=0.390; p=0.001) and disease severity (HS-PGA≥3) (r=0.639; p<0.0001), once adjusting for age, sex and BMI. However, no correlation was found between plasma ghrelin concentrations and clinical or laboratory parameters.

Circulating RBP4 levels were significantly higher in patients with HS-PGA score≥3 than in those with minimal-mild HS (HS-PGA score<3)(67.7 [60.5-77.5] vs. 47.0 [45.6-55.1];p<0.0001). However, no significant differences were observed in ghrelin concentrations between both HS-PGA groups (56.1 [50.0-185.9] vs. 50.0 [50.0-189.7]; p=0.73).

4.3 Influence of RBP4 and ghrelin on the risk for HS

Table 2 shows the results of the logistic regression analysis of independent variables considered to influence HS risk, adjusted by age, sex,BMI, IR, and active tobacco use. Thus, raised serum levels of RBP4 (above the median), and lower levelsof ghrelin (below the median) were related to an increased risk for HS development (OR 14.50[CI 95%, 4.55-46.19]; p<0.0001, and OR 3.86 [CI 95%, 1.32-11.27]; p=0.013, respectively). The inclusion of additional covariables, such as serum hs-CRP levels, BP or MS to the regression model did not virtually change theseresults.

5 CONCLUSIONS

This is the first study, to our knowledge, that assesses RBP4 and ghrelin concentrations in patients with HS. We found that serum RBP4 levels were significantly increased in HS-patients and positively correlated with disease severity. In this regard, increased plasma levels of this adipokine have also been found in other cutaneous diseases, such as contact dermatitis [21]and psoriasis. [22]Moreover, raised RBP4 concentrations in our study were associated with an increased risk for HS, suggesting that this adipokine might play a role in the pathogenesis of HS.In this respect, it should be noted that RBP4 acts as an immunomodulatory adipocytokine and may induce the release of pro-inflammatory mediators, including TNF-α, IL-1β and IL-6.[12]Furthermore, in our HS-patients, serum RBP4 levels were positively associated with IR even after adjustment for BMI, suggesting that other factors independent of obesity might be implicated in such an association. It is known that RBP4 may inhibit insulin signaling in adipocytes by inducing the release of classic pro-inflammatory mediators from macrophages.[15]These factssuggest that RBP4 might represent a mechanistic link between the pathological inflammatory process of HS and the development of IR in patients with this disease.

On the other hand, ghrelin is a pleiotropic peptide-hormone/cytokine that exerts an anti-inflammatory function,[16,23] although its production may also be directly inhibited by pro-inflammatory mediators.[24]In our study, serum ghrelin levels were significantly lower in HS-patients than in controls, irrespective of BMI. In this regard, the pro-inflammatory cytokines implicated in the chronic inflammatory process of HS might have played a role in the lower serum ghrelin levels.Regarding the association between ghrelin and IR, several

studies have found an inverse correlation.[25]However, we did not find any relationship between serum ghrelin levels and IR in HS-patients. In this sense, it is tempting to speculate that ghrelin secretion in HS patients might be influenced by an increase of pro-inflammatory cytokines, leading to a decrease in its serum levels.Besides, we have also found that serum ghrelin levels were negatively related to the risk for developing HS, although we cannot explain this protective finding through a beneficial effect on IR.

In conclusion, we found that serum RBP4 levels were significantly increased and serum ghrelin concentrations significantly decreased in HS-patients.Furthermore,our results suggest that high RBP4 levels may be a surrogate biomarker for IR in patients with HS. Finally,increased serum RBP4 and decreased ghrelin levels could be independent risk factors for the development of HS.

Conflict of interest: None declared

Authors contributions:

MAGL recruited patients for the study, contributed to the elaboration of the protocol of study, performed the study, helped in the interpretation of data and was responsible of the final drafting and elaboration of the manuscript. GOV performed the study, contributed to the elaboration of the protocol of study, helped in the interpretation of data and in the elaboration of the manuscript. CM contributed to the elaboration of the protocol of study, helped in the interpretation of data and contributed to the elaboration of the manuscript. IV performed the study, helped in the interpretation of data and in the elaboration of the manuscript. SG performed the study and helped in the interpretation of data. VP performed the study and helped in the interpretation of data. RB performed the study, contributed to the elaboration of the protocol of study, helped in the interpretation of the data and in the elaboration of the manuscript. JLH contributed to the elaboration of the protocol of study, helped in the interpretation of data and was responsible of the final drafting and elaboration of the manuscript.

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Parameter	HS patients (n=77)	Controls (n=60)	p
Age, *yrs*	42.7 ± 11.7	45.7 ± 13.0	0.16
Sex, *male (%)*	48.1	51.7	0.67
Active smoking, *%*	66.2	18.3	**<0.0001**
BMI, *Kg/m²*	29.5 ± 5.4	26.6 ± 4.5	**0.001**
Waist perimeter, *cm*	99.9 ± 13.7	91.7 ± 13.7	**0.001**
SBP, *mm Hg*	133.1 ± 15.7	124.8 ± 15.6	**0.002**
DBP, *mm Hg*	82.4 ± 13.6	77.3 ± 8.1	**0.012**
hs-CRP, *mg/dl*	0.42 (0.18-0.89)	0.10 (0.10-0.20)	**<0.0001**
HbA1c, *%*	5.2 ± 0.6	5.2 ± 0.3	0.63
LDL-c, *mg/dl*	116.3 ± 32.5	122.9 ± 29.2	0.22
HDL-c, *mg/dl*	46.0 (41.5-56.5)	52.5 (46.3-70.5)	**0.001**
Triglycerides, *mg/dl*	100.3 ± 47.7	98.1 ± 66.7	0.82
Fasting plasma glucose, *mg/dl*	94.6 ± 13.8	89.1 ± 8.1	**0.004**
Fasting plasma insulin, *μIU/ml*	10.8 (5.7-17.2)	7.5 (5.0-10.8)	**0.007**
HOMA-IR	2.3 (1.1-3.8)	1.5 (0.9-2.3)	**0.006**
Insulin Resistance, %	46.8	20.0	**0.001**
Hypertension, *%*	18.2	15.0	0.62
Dyslipidemia, *%*	13.2	16.7	0.57
Metabolic Syndrome, %	32.5	11.7	**0.004**
Ghrelin, *ng/ml*	50.0 (50.0-186.3)	210.5 (117.7-302.9)	**<0.0001**
RBP4, *μg/ml*	59.3 (47.2-72.7)	38.5 (34.7-44.3)	**<0.0001**

Table 1. Demographic, clinical and laboratory findings of patients with HS and controls

BMI, body mass index; HbA1c, glycated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure; hs-CRP: high-sensitive C-reactive protein; LDL-c, low-density lipoprotein; HDL-c, high-density lipoprotein; HOMA-IR, Homeostatic model assessment for insulin resistance. RBP4, retinol binding protein 4. Values are expressed as mean ± SD or median (interquartile range) as appropriate.

	β-coefficient	OR (CI 95%)	*p*
Age, *yrs.*	-0.060	0.94 (0.90-0.98)	0.007
Active smoking, *yes*	2.882	17.85 (5.32-59.82)	<0.0001
Insulin Resistance, *yes*	1.656	5.24 (1.55-17.72)	0.008
Ghrelin *(median), ng/ml*[*]	1.351	3.86 (1.32-11.27)	0.013
RBP4 *(median),μg /ml*[**]	2.683	14.50 (4.55-46.19)	<0.0001

Table 2. Adjusted risk factors for HS development.

[*] *Ghrelin levels below median (<130 ng/ml).* [**] *RBP4 levels above the median (>47.3 ng/ml)*