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PROGRAMA DE MAESTRÍA Y DOCTORADO EN CIENCIAS MÉDICAS, ODONTOLÓGICAS Y DE LA SALUD

"ADIPOCITOCINAS EN GOTA, SU RELACION CON EL ESTADO CLINICO Y CON EL SINDROME METABOLICO"

TESIS

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MAESTRO EN CIENCIAS MÉDICAS

PRESENTA

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“A prospective follow-up of adipocytokines in a cohort of patients with gout. Association with Metabolic syndrome and not with clinical inflammatory findings.

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FIRMAS DE APROBACIÓN

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TESIS PARA OBTENER EL GRADO DE MAESTRO EN CIENCIAS MÉDICAS.

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La tesis fue realizada por el alumno Sergio García Méndez, y se entrega en la modalidad de artículo, el resumen del mismo fue enviado para evaluar su publicación en la revista Medicine (se anexa el comprobante de evaluación del articulo).

Como primer autor, el Dr. Sergio García Méndez realizó la parte medular de este trabajo con la ayuda de los demás colaboradores.

De acuerdo a la política editorial de dicha revista, el número de palabras tanto en el “Abstract” como en el cuerpo del manuscrito, el número de figuras y tablas se ajustara a su normativa.

La tesis por lo tanto se presenta en el formato en que fue enviada a publicación.

Atentamente,

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CONTENTS

Abstract

Objective: To determine the levels of leptin (Lep) and adiponectin (AdipoQ) in patients with gout and its relationship with joint inflammatory data and/or with metabolic syndrome (MetS) variables, during one year follow-up.

Methods: Forty one patients (40 males) with gout diagnosis, attending for the first time to a rheumatology department were included. Evaluations were performed baseline, at 6 and 12 months. Variables included: Demographic, clinical and laboratory data related to gout and associated diseases. Lep and AdipoQ determinations by ELISA method were performed in frozen serum from each visit. The pharmacological and no-pharmacological treatment for gout and associated diseases was individualized for each patient according to published guidelines. Statistical analysis included Mann-Whitney U test, Fisher's test, x^2, ANOVA, Cochran´s Q, Pearson and Spearman correlation tests as well as lineal and logistic regression.

Results: In the baseline evaluation, 29.2% had MetS (hypertriglyceridemia 66%, hypertension 44% and obesity 37%); patients with MetS had higher C reactive protein (CRP) levels [34.1 ±28.6 VS 12.2 ±11.2 mg/dL, p=0.033]. Although not significant, also had higher Lep and lower AdipoQ levels (3.2 ± 3.0 VS 1.9 ±1.2 ng/mL, p=0.142 and 40.5 ±26.8 VS 38.0 ±24.9 ng/mL, p=0.877 respectively). During follow-up, our patients had significant improvement in serum uric acid (sUA) levels and variables evaluating pain and joint swelling (p≤0.05). Metabolic abnormalities tended to persist or even worsen during the monitoring period:

significant increase in total cholesterol (p=0.004), tendency to higher triglycerides (p=0.883) and slight improvement in glycaemia (p=0.052). Lep values increased significantly during follow-up (p=0.001) while AdipoQ levels diminished slightly (p=0.317). Neither Lep nor AdipoQ values showed important correlation (r>0.5) with metabolic variables or joint swelling.

Conclusion: This study suggests that in patients with gout, concentrations of Lep and AdipoQ are more in line with the metabolic state than with clinical disease activity.

Key words: gout, leptin, adiponectin, metabolic syndrome, obesity.

Introduction

Adipocytokines are proteins secreted by white adipose tissue, among which leptin (Lep) and adiponectin (AdipoQ) have been associated with regulatory functions on energy metabolism and implicated as mediators of systemic inflammatory responses as well as promoting a “low degree” inflammatory status and mediating immune responses [1-3].

These two adipocytokines are closely related to obesity and/or resistance to insulin and are important in the development of metabolic syndrome (MetS) and its components [4-6]. In this context, a positive correlation between serum uric acid (sUA) and Lep levels has also been reported [7-9], as well as negative correlation with AdipoQ [10, 11].

The role that they play in different rheumatic diseases has recently been under study and, in spite of inconsistent results; a pro-inflammatory role has been described in osteoarthritis (OA) and in rheumatoid arthritis (RA) [12-16].

In RA, results have been contradictory, as Lep levels may be higher than in healthy controls [12] or even similar [13], related to the severity of RA [17-19] and the serum/synovial fluid, Lep gradient is related to the time since onset of disease and disease activity [20]. On the other hand, Lep has a negative correlation with radiological damage [21] and in fasting conditions, may promote an anti-inflammatory milieu [22]. High concentrations of AdipoQ are promoters of the expression of inflammatory molecules [14, 23]; however, their presence has also been related to the inhibition of inflammatory pathways and immune response mediated by tumor necrosis factor α (TNFα) action [24].

With regard to OA, it has been recently shown that joint tissue secretes greater amounts of Lep and this has been related with greater structural damage mediated by proinflammatory cytokines and matrix metalloproteinases [12, 15, 25-27]. The role of AdipoQ in OA is controversial; on the one hand its serum concentration is significantly higher than in healthy controls [28], related to severity [29] and joint inflammation [30], while on the other there is evidence that links it with less clinical and radiological damage [31].

There are few studies in patients with gout in which the role of these adipocytokines had been studied [32-34], documenting a significant increase of AdipoQ in patients who received benzbromarone [32, 34]; apparently, this increase in AdipoQ is produced by an agonist effect on peroxisome proliferator-activated receptors gamma (PPARγ) with anti-inflammatory consequences [32]. In a case control study it was observed that the serum concentrations of Lep and AdipoQ were related to the body mass index and the amount of abdominal fat [33]. It must be pointed out that none of these studies evaluated the relationship between these adipocytokines and the reduction in joint inflammatory activity of patients.

The objective of the present study was to determine the levels of Lep and AdipoQ in patients with gout under regular treatment and its relationship with the presence of MetS and joint inflammatory activity.

Patients and methods

This was an observational and prospective study nested in the GRESGO (GRupo de EStudio de GOta) cohort, that includes patients with gout diagnosis (according to ACR and CGD criteria) [35, 36], whom attended to the rheumatology department

of our hospital for the first time. The project was approved by the local ethics and research committees and in the baseline visit all patients signed an informed consent form.

They were evaluated clinically by one of the participating rheumatologists (SGM, EAH or JVM) and received verbal and written information about gout and associated diseases.

The prescription in the baseline and subsequent visits included 1) Lifestyle modifications: Diet, exercise and weight reduction and 2) Pharmacological treatment including allopurinol or other urate lowering drug (ULD), non-steroidal anti-inflammatory drugs (NSAID), steroids, colchicine and treatment for associated diseases, according to published recommendations for gout treatment (37, 38).

The visits were performed as frequent as the clinical status of each patient required but all them attended at least to the baseline, 6 and 12 months visits. The evaluation included tender, swollen and limited to motion joint count; the number of acute flares in the last six months, clinimetric evaluation (HAQ questionnaire, VAS for pain and global health).

During the patient regular visit to the laboratory for biochemical determinations, 1 mL of serum was frozen at -70°C until adipocytokine determination. It was performed through indirect ELISA using a human anti-Leptin antibody (Santa Cruz Biotech Inc.), human recombinant Leptin (PeproTech Inc.), anti-adiponectin antibodies (Santa Cruz Biotech Inc) and human recombinant adiponectin (R&D Systems).

The presence of MetS and its associated entities was determined according to the criteria proposed by the Adult Treatment Panel III (ATP III) [39]: obesity (waist

circumference ≥88 cm in women or ≥102 cm in men), dyslipidemia (high density lipoprotein-cholesterol (HDL-C) ≥40 mg/dL in men or ≥50 mg/dL in women, and triglycerides ≥150 mg/dL), hyperglycemia defined as fasting glucose ≥110 mg/dL or type 2 diabetes mellitus (DM2) [40], hypertension (≥130/85 mmHg or if under treatment). Mean arterial pressure (MBP) = ((2 x diastolic arterial pressure) + systolic arterial pressure) / 3.

Glomerular filtration rate (GFR) was determined through a 24-hour creatinine clearance test and using the "Modification of Diet in Renal Disease" formula (MDRD) (GFR = 186 x (creatinine) -1.154 x (age) - 0.203 or (x 0.742 in women)) [41]. Chronic renal failure (CRF) was assumed when the GFR was ≤60 mL/min/1.73 m^2. All of the determinations were done in triplicate in the three evaluations and were reported as means and standard deviations.

For the statistical analysis we employed Mann-Whitney U test, Fisher's test, x^2, ANOVA, Cochran´s Q, Pearson and Spearman correlation tests as well as lineal and logistic regression. All statistical tests were performed using IBM SPSS Statistics 21.

Results

Forty-one patients with gout, mean age of 48.0 ± 12.9 years were included. Forty of them were males; thirty two (78.1%) had tophaceous gout and in 80.5% of the patients we demonstrated monosodium urate crystals upon polarized light microscopy of the synovial fluid or tophi.

At baseline, the mean duration of disease was 12.6 ±10.5 years; however, none of them received adequate treatment and therefore had evidence of disease

inflammatory activity (frequent acute flares in the 6 months prior to baseline visit and swollen and tender joints as well as an increase in acute phase reactants) and also chronicity data (hyperuricemia, tophi and limited to motion joints). Almost one third of the patients had MetS or its comprising entities, the most common of which was hypertriglyceridemia, followed by hypertension and obesity; a third of the patients had CRF (Table 1).

Baseline evaluation: Gout with MetS VS Gout without MetS

In the baseline evaluation, patients with gout and MetS had significantly greater levels of CRP and greater but not significantly levels of Lep. On the other hand, these same patients had a better renal function according to the three measures we employed for this variable (MDRD, serum creatinine and percentage of patients with CRF), but only serum creatinine levels had a tendency to be significantly different (Table 2).

As expected, when comparing patients with MetS to those without it, the former had a greater frequency of hypertension, obesity, hyperglycemia and dyslipidemia; however, we did not find significant differences in the related demographic or clinical variables related to gout or in the AdipoQ levels (Table 2).

6 and 12 months follow up

Gout

We found significant improvement in the sUA values, MDRD and in the variables evaluating joint pain and swelling at 6 and 12 months (Figures 1a/1b). We observed that patients with MetS had higher baseline levels of CRP and at 6

months; these values steadily decreased, although this change was not significant (Table 3).

After one year of follow up, only 10 patients (24.4%) had sUA levels ≤6.0 mg/dL; treatment was individualized but in general terms consisted of starting regular treatment with allopurinol at a low dose followed by a steady increase: (300 ±189.8 mg/day at the baseline visit to 450 ± 206 mg/day at 12 months). Forty patients received allopurinol and only one patient received probenecid (due to allergy to allopurinol); all patients received prophylaxis with colchicine (1 mg/day).

MetS

During the follow up, we did not find significant improvement in the associated metabolic entities, our patients showed increase in total cholesterol and tryglicerides, maintained their waist circumference and MBP, while discretely improved HDL-C and glycaemia (Table 3). Although 57.9% of them (MetS patients) received bezafibrate, 5.1% fenofibrate, 10.5% received statins, 73.7% losartan or enalapril and metformin was prescribed to 26.3%. Enalapril or losartan were employed as antihypertensive treatment in those who required it, only one patient received diuretics and another was taking low dose aspirin.

Clinical status and serum adipocytokine levels

The levels of adipocytokines, especially Lep, tended to increase both in the group of patients with MetS as well as overall, in the same way that metabolic abnormalities tended to persist or even worsen during follow-up (Table 4).

After performing a linear correlation of the Leptin and AdipoQ values, there was no important correlation ($r>0.5$) between other metabolic variables and those measuring the activity of gout.

During follow-up, a 54-year-old patient died due to a myocardial infarction; this patient had a history of intense tobacco and alcohol consumption, tophaceous gout for 30 years with an inadequate control, MetS (obesity, hypertension and dyslipidemia), his baseline Lep and AdipoQ serum levels were similar to the values from other patients.

Discussion

This study evaluated the possible relationship between improvement in clinical gout and/or metabolic data and the serum concentration of adipocytokines in patients in whom regular treatment was initiated.

During follow-up with regular treatment, the patients improved significantly in all the variables directly related to gout as joint inflammatory activity and in the sUA levels; although only one fourth of them achieved sUA ≤6.0 mg/dL. Similar to other studies, we did not find relation among the concentration of adipocytokines and sUA levels [32, 33].

Lep values had relationship with obesity, MetS and CRP, but not with gout related variables and course during 6 and 12 months follow-up. These Lep and AdipoQ (especially Lep) values related to MetS course had been previously reported in patients with MetS, without gout [42-45].

Interestingly CRP, a marker of systemic inflammatory state and considered a marker for joint “activity” as well as marker for metabolic and cardiovascular risk,

tended to lower levels during follow-up in patients with gout associated to MetS, but not in patients without it.

Previous reports evaluating whether adipocytokines are protective or harmful in joint inflammatory diseases, had been controversial. However, at the same time, some authors have reported that the presence of high levels of these adipocytokines must always be considered abnormal and may be associated with metabolic or inflammatory abnormalities; in addition, the diversity of cells containing receptors for these adipocytokines make them scarcely specific and finally, their expression and functions seem to be different in different body tissues [46].

Unfortunately, we were not capable to modify most metabolic variables during one year of follow-up. The data are unsatisfactory because, excepting a tendency to lower glucose levels, the rest of the variables did not improve and there was even a significant increase in the total cholesterol levels; in addition, during follow up we observed increase in the number of patients with low HDL-C values and MetS.

There are difficulties in compliance and adherence in patients with chronic diseases and especially in gout patients, in one study >80% of gout patients had partial and poor adherence to ULD therapy, the adherence to lifestyle modifications in them seems to be lesser, although not known [47].

Probably the sample size of this study, although adequate to evaluate clinical improvement, was not enough to evaluate minor changes in AdipoQ values. Finally, all the above results suggest that in patients with gout, the concentrations of Lep and AdipoQ are mainly related to the metabolic state and not the articular clinical activity itself.

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Table 1. Baseline demographic and clinical data.

	n = 41
Demographic variables.	
Males/females; n.	40/1
Current age, years.	48.0 (12.9)
Age at onset, years.	34.6 (13.9)
Metabolic syndrome variables, n (%).	
Hypertriglyceridemia.	27 (65.9)
Hypertension.	18 (43.9)
Obesity.	15 (36.6)
Low HDL-C.	14 (34.1)
Metabolic syndrome	12 (29.3)
Hyperglycemia.	8 (19.5)
Type 2 diabetes mellitus	4 (9.8)
Renal function variables.	
MDRD, mL/min/1.73 m^2.	76.1 (28.4)
Creatinine, mg/dL.	1.2 (0.4)
Chronic renal failure, n (%).	13 (31.7)
Adipocytokines values.	
Leptin, ng/mL.	2.3 (2.0)
Adiponectin, ng/mL.	39.2 (25.2)
Gout associated variables.	
Acute flares in the last 6 months.	3.8 (6.4)
Number of painful joints.	3.2 (5.7)
Number of swollen joints.	0.6 (1.6)
Number of limited to motion joints.	5.9 (9.9)
Number of tophi.	8.6 (9.9)
Tophaceous gout; n (%).	32 (78.1)
Serum uric acid, mg/dL.	8.3 (2.2)
C reactive protein, mg/L.	17.6 (19.1)

Values are the mean and standard deviation (SD) unless specified.
HDL-C: High density lipoprotein-cholesterol; MDRD: Modification of diet in renal disease.

Table 2. Comparison of baseline demographic and clinical data of gout patients with or without metabolic syndrome.

	With Metabolic Syndrome (n=12)	Without Metabolic Syndrome (n=29)	p
Demographic variables			
Current age, years.	46 (12.3)	51 (14.2)	NS[£]
Age at onset, years.	32.6 (16)	35.7 (12.7)	NS[£]
Renal function variables.			
MDRD, mL/ min/m^2.	82.7 (29.7)	73.4 (28.0)	NS[£]
Creatinine, mg/dL.	1.0 (0.2)	1.3 (0.4)	0.060[£]
Chronic renal failure, n (%).	3 (25.0)	10 (34.5)	NS[§]
Adipocytokines values			
Leptin, ng/mL.	3.2 (3.0)	1.9 (1.2)	0.142[£]
Adiponectin, ng/mL.	38.0 (24.9)	40.5 (26.8)	0.877[£]
Gout associated variables			
Acute flares in the last 6 months.	3.6 (3.9)	3.8 (7.3)	NS[£]
Number of painful joints.	5.1 (7.1)	2.4 (4.9)	0.056[£]
Number of swollen joints.	1.2 (0.9)	0.4 (0.2)	NS[£]
Number of limited to motion joints.	7.0 (5.9)	5.4 (3.8)	NS[£]
Number of tophi.	10.2 (8.2	7.9 (6.3)	NS[£]
Tophi; n (%).	10 (83.3)	22 (75.9)	NS[§]
Serum uric acid, mg/dL.	8.3 (2.1)	8.3 (2.3)	NS[£]
C reactive protein, mg/L.	34.1 (28.6)	12.2 (11.2)	0.033[£]

Values are the mean and standard deviation (SD) unless specified.
MDRD: Modification of diet in renal disease.
[£] Mann-Whitney U test; [§] Chi square.

Table 3. Comparison of metabolic variables. Baseline, 6 and 12 months in patients with or without metabolic syndrome and in the whole group.

	With metabolic syndrome			Without metabolic syndrome			Whole Group		
	Baseline	**6 m**	**12 m**	**Baseline**	**6 m**	**12 m**	**Baseline**	**6 m**	**12 m**
Waist circumference, cm	108	109.0	107.6	96.2	96.7	97.0	99.8	100.3	100.1
	(9.6)	(8.3)	(7.9)	(8.5)	(8.5)	(8.2)	(10.4)	(10.1)	(9.4)
Mean blood pressure, mmHg	93.3	100.1	99.1	91.3	92.2	90.7	91.9	94.5	93.1
	(8.9)	(9.6)	(11.4)	(11.1)	(9.5)	(10.3)	(10.4)	(10.1)	(11.2)
Glucose[§], mg/dL	111.5	100.0	99.7	92.6	92.0	90.2	98.1	94.3	93.0
	(20.2)	(17.1)	(15.6)	(15.5)	(9.2)	(10.9)	(18.9)	(12.4)	(13.0)
Triglycerides, mg/dL	229.3	297.0	277.5	227.2	206.5	237.1	227.8	234.3	249.8
	(127.1)	(267.0)	(127.5)	(126.8)	(82.6)	(136.1)	(125.2)	(163.4)	(135.0)
HDL-C, mg/dL	30.8	34.6	36.2	38.6	37.3	51.3	34.9	36.4	38.4
	(10.1)	(11.2)	(7.5)	(10.4)	(6.9)	(77.1)	(10.8)	(9.3)	(9.4)
Cholesterol*, mg/dL	177.2	184.1	193.9	175.6	187.4	186.6	176.0	187.2	191.6
	(31.2)	(25.5)	(26.9)	(35.2)	(35.1)	(38.0)	(33.7)	(32.9)	(33.4)
C reactive protein[£], mg/L	34.1	23.0	9.8	12.2	28.0	13.3	17.6	26.8	12.4
	(28.6)	(24.3)	(6.6)	(11.2)	(40.0)	(14.4)	(19.1)	(35.9)	(12.8)
Leptin, ng/mL	3.2	2.8	4.3	1.9	2.3	2.9	2.3	2.4	3.3
	(3.0)	(2.6)	(2.6)	(1.2)	(1.4)	(1.3)	(2.0)	(1.8)	(1.8)
Adiponectin, ng/mL	40.5	35.9	38.5	38.7	36.8	32.7	39.2	36.5	34.4
	(26.8)	(21.3)	(27.9)	(24.9)	(25.0)	(21.2)	(25.2)	(23.7)	(23.1)

Values represent the mean and standard deviation (SD). HDL-C: High density lipoprotein-cholesterol.

Significant p values: [§]Glucose baseline VS 12 months in the whole group, p=0.052; *Cholesterol, patients with metabolic syndrome baseline VS 6 months: p= 0.020 and baseline VS 12 months: p=0.030; Cholesterol in the whole group, baseline VS 12 months: p=0.004; [£]C reactive protein, patients with metabolic syndrome + baseline VS 12 months: p=0.097; Leptin values were statistically different in: 1) In patients with metabolic syndrome were different in 6 VS 12 months evaluation: p=0.026. 2) In patients without metabolic syndrome were different in the three evaluations: Baseline VS 6 months: p=0.057, 6 VS 12 months p=0.015 and baseline VS 12 months, p=0.001. 3) In the whole group: Baseline VS 12 months and 6 VS 12 months: p=0.001 for both (ANOVA).

Table 4. Percentage of patients with Gout with associated diseases.

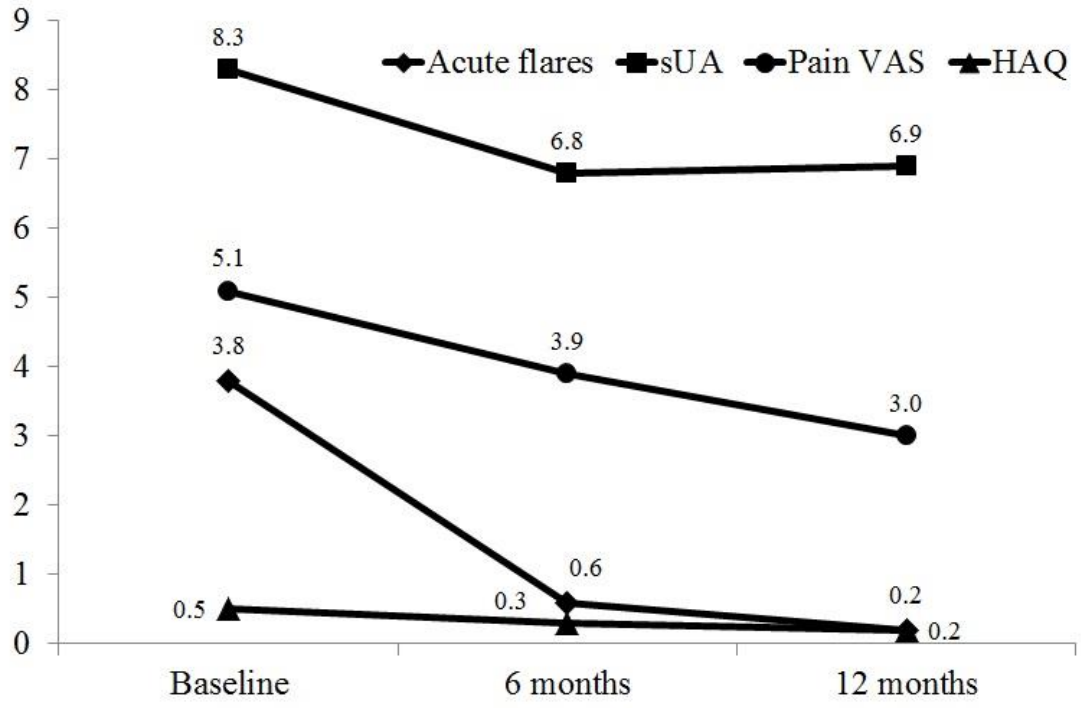
Associated diseases	Baseline	6 months	12 months	p*
Obesity[§]	15 (36.5)	19 (46.3)	18 (43.9)	0.156
Hypertension[§]	18 (43.9)	19 (46.3)	19 (46.3)	0.368
Hyperglycemia[§]	8 (19.5)	5 (12.1)	3 (7.3)	0.093
Hypertriglyceridemia[§]	27 (65.8)	28 (68.2)	34 (82.9)	0.037
HDL-C low[§]	14 (34.1)	27 (65.8)	26 (63.4)	0.002
Metabolic syndrome[§]	12 (29.2)	17 (41.4)	19 (46.3)	0.050
Hypercholesterolemia	7 (17.0)	12 (29.3)	15 (36.5)	0.076
Chronic renal failure	13 (31.7)	7 (17.0)	7 (17.0)	0.011
Ischemic heart disease	0 (0.0)	0 (0.0)	1 (2.4)	0.368

Values represent n (%).

[§]ATP III metabolic syndrome criteria

*Cochran Q.

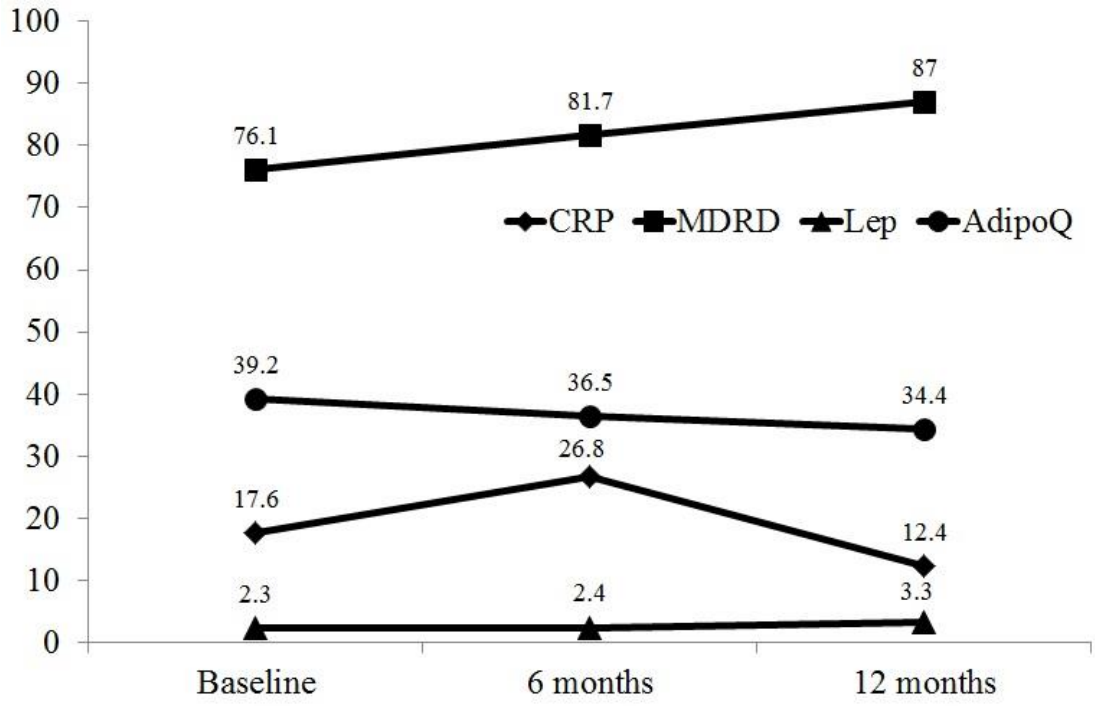
Figure 1a. Improvement in Gout clinical data (mean values). Baseline, 6 and 12-month evaluations.



Footnote (Figure 1a):

Acute flares: Number of flares in the last 6 months; sUA: Serum uric acid (mg/dL); VAS: visual analogue scale; HAQ: Health assessment questionnaire score.

Figure 1b. Gout para-clinical data and Adipocytokine levels (mean values). Baseline, 6 and 12-month evaluations.



Footnote (Figure 1b)

CRP: C reactive protein (mg/dL); MDRD: Modified diet in renal disease (mL/min/1.73 m^2); Lep: Leptin (ng/mL); AdipoQ: Adiponectin (ng/mL).

REQUEST FOR REVISION

Apr 02 2015 10:05AM

RE: MD-D-15-00549, entitled "A prospective follow-up of adipocytokines in a cohort of patients with gout. Association with Metabolic syndrome and not with clinical inflammatory findings."

Dear MD PhD Vazquez-Mellado:

Your manuscript has been carefully reviewed, and the reviewer/editorial comments and queries are listed below. Revision and response is necessary before the paper can be considered for further review.

Please submit the revised manuscript via Editorial Manager by May 14 2015 11:59PM. If you are unable to revise within this time, please contact the Editorial Office with a request for an extension or your paper will be removed from the editorial process. A request for an extension must be submitted within 60 days of the date of this letter.

All revised manuscripts must include an itemized, point-by-point response to each reviewer, including the reviewer's original comment(s). Specify the changes made to address each of their concerns; include the changes made in the response or indicate their locations in the manuscript. Address each reviewer comment using

the reviewer's number. Submit this "Response to Reviewers" as a separate document when uploading the requisite files in the system.

* If your manuscript is accepted for publication, payment of the article processing charge (APC) must be completed by visiting: http://wolterskluwer.qconnect.com. Upon entering the site for the first time, authors will be prompted to create a user ID and password. The APC for *Medicine*® is $1,200. The publication fee must be paid within 30 days of article acceptance by credit card by the author, funding agency, or institution. Payment must be received in full for the article to be published.

To submit your revision, go to http://www.editorialmanager.com/md and login as an Author. Click on the menu item "Submissions Needing Revision" to obtain your submission record and begin the revision process.

We look forward to receiving your revised manuscript.

Best Regards,

Patrick Wall

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