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Ghrelin level as a biomarker for knee osteoarthritis severity and appearance in HIV + patients

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ABSTRACT

Background: Knee Osteoarthritis (KOA) is a multifactorial disease with several mechanisms to promote articular cartilage damage. New molecules, such as ghrelin, have been recently reported to participate in the pathogenesis and progression of KOA. In HIV + patients, arthralgias are the most frequent musculoskeletal manifestations, mainly affecting joints such as the knee. Also, it has been reported that HIV + patients have a reduction of ghrelin even with treatment compared to HIV- patients. However, there is no report in the literature evaluating ghrelin and KOA in the HIV + population. We aimed to evaluate whether serum ghrelin levels can function as a biomarker for OA in HIV + patients.
Methods: We recruited 40 patients, 20 HIV+, and 20 HIV- controls, and grouped as follows: HIV+/KOA+; HIV+/KOA-; HIV-/KOA+; HIV-/KOA-. Clinical features were obtained during clinical visits. Peripheral blood samples were acquired to measure serum ghrelin levels.
Results: The HIV+/KOA + group significantly reduced serum ghrelin levels when compared with the other groups. Comparing the ghrelin levels with the patients' nadir of $CD4^+$ T-cells count, we identified a statistically significant negative correlation in the KOA- group ($r = -0.80$, $P < 0.007$). An ROC curve analysis, for the accuracy of ghrelin levels to identified HIV+/KOA + from HIV+/KOA- patients, found an area under the curve of 0.83 (95 % CI 0.65–0.10; $P = 0.017$), with a cut-off < 4026 pg/mL serum ghrelin levels, with a sensitivity of 0.62 (95 % CI 0.32–0.86), and a specificity of 0.10 (95 % CI 0.59–0.10).
Conclusion: This study shows the potential use of ghrelin levels as a biomarker for KOA in the high-risk HIV population that should be further analyzed.

Abbreviations: OA, Osteoarthritis; KOA, Knee Osteoarthritis; HIV, Human Immunodeficiency Virus.

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1. Introduction

The survival of people living with human immunodeficiency virus (HIV) (PLWH) has increased thanks to antiretroviral therapy; however, new challenges have emerged, such as the increase of musculoskeletal disorders, such as osteoarthritis (OA) [1]. OA is a chronic degenerative pathology characterized by an inflammatory component that causes loss of articular cartilage and changes in the synovial membrane [2,3]. Worldwide, OA is considered as one of the main causes of disability in people > 65 years old, affecting around 50 % of this population, and the knee is the most affected [4].

Currently, several risk factors have been confirmed to facilitate OA appearance and progression. The major risk factors include age and female gender, followed by overweight and obesity with a relative risk of 2 and 2.96, respectively [5]. In addition, the metabolic syndrome has been related to a 2.3–9.7 increased risk for knee OA (KOA), depending on how many components of the metabolic syndrome are present in the patient [6]. Furthermore, the surgical procedures and articular damage – but not the physical activity itself – are known risk factors for KOA, increasing it up to five to seven times [7]. HIV-positive (HIV +) patients are another major group afflicted by arthralgias, with up to 40 % of patients having symptoms and the knee being one of the most affected joints [8]. However, most of the KOA research has been carried out without directly evaluating this population.

Nowadays, the involvement of new molecules in bone homeostasis has been evaluated, as in the case of ghrelin [9,10]. Mainly known as a hunger hormone, ghrelin has recently been reported to participate in bone and cartilage metabolism by stimulating the proliferation, differentiation, and migration of several cell lines, including osteoblasts and chondrocytes [9,10]. Also, it has been reported that ghrelin is involved in inflammatory pain, mechanical hyperalgesia, and chronic neuropathic pain [11,12]. Lastly, ghrelin has been suggested to have a protective role in the pathogenesis and progression of KOA [13,14].

In HIV + patients, serum ghrelin levels have been shown to be decreased and antiretroviral therapy increases them, yet they remained lower in comparison to non-infected patients [15]. However, there are no reports evaluating the effects of the reduction of ghrelin in the context of KOA in HIV + patients. In this study, we aimed to evaluate ghrelin and its potential as a biomarker for KOA in HIV + patients.

2. Material and methods

2.1. Sample acquisition

A descriptive, transversal, prospective, analytic study including 20 HIV + patients and 20 HIV negative (HIV-) control patients was performed. HIV + patients were recruited from the HIV clinic and the control patients from the Orthopedics Trauma Service of the tertiary care university hospital 'Hospital Civil de Guadalajara – Fray Antonio Alcalde' (a 1000-bed teaching hospital in Western Mexico) from February to September 2018. Selection criteria were male or female HIV + or HIV- patients, between 30 and 75 years old, body mass index (BMI) < 30, with or without KOA, without other knee pathology, and no history of previous knees surgeries. Volunteers were invited and signed voluntarily to participate in the study. The project was approved by the ethics committee in research of the Hospital Civil de Guadalajara' Fray Antonio Alcalde' with registry number 095/18. Procedures were fulfilled in accordance with 2013′s Helsinki Declaration [16].

Selected patients were grouped into four groups: HIV + and KOA+; HIV + and KOA-; HIV- and KOA+; HIV- and KOA-.

2.2. Patient evaluation

An orthopedic specialist checked recruited patients and performed the clinical history and medical exploration, including somatometry. Afterwards, knee radiographs were taken, and the Kellgren and Lawrence classification was used to assessed KOA. Radiographic KOA was defined as Kellgren and Lawrence grade of ≥ 2. Lastly, Lysholm Knee Scoring System was performed to evaluate knee dysfunction severity.

2.3. Serum collection

After clinical evaluation, a peripheral veinous blood sample was taken from all patients. Samples were acquired in two serum-separating tubes (BD, New Jersey, USA), centrifuged in a high-speed Z36HK centrifuge (HERMLE Labortechnik, Wehingen, Germany) at 2290*g* at 20 °C for 20 min and serum was collected in 500-μL aliquots and stored at −80 °C.

2.4. Ghrelin quantification

Once all serum samples were acquired, serum ghrelin levels were analyzed using the 'Human Ghrelin' ELISA Kit (CUSABIO cat# CSB-E13398h, TX, USA). The kit has a detection limit of 0.625–8000 pg/mL and a sensibility of 0.156 pg/mL. Analysis was performed following manufacturer instructions, and samples were measured using a multi-modal reader 'Synergy' (BioTek, VT, USA).

2.5. Statistical analysis

Proportions were compared using the Chi-squared test or Fisher's exact test. Group comparisons for numerical variables were performed using the Mann–Whitney U-test or Kruskal–Wallis test with Bonferroni–Dunn correction, depending on the groups' number. For additional analysis, receiver operating characteristic (ROC) curve and Spearman's correlations were performed. All statistics were calculated using IBM SPSS Statistics for Windows version 22 (IBM Corp., Armonk, NY, USA); plots were drawn with GraphPad Prism version 8 (GraphPad Software, La Jolla, CA, USA). Differences in biomarker levels or correlations were considered statistically significant if the *P*-value was < 0.05.

3. Results

We recruited 40 patients, 20 with HIV and 20 non-HIV controls, from which 13 and 7 were KOA+, respectively. We separated the patients into four groups: HIV+/KOA+, HIV+/KOA-, HIV-/KOA+, and HIV-/KOA-. With an overall average age of 43 years, the most common gender was female; all groups had similar BMI with a tendency of overweight in HIV+/KOA + and HIV-/KOA-. In the HIV group, 70 % of the patients KOA + and 60 % of KOA- had comorbidities. The most common comorbidities in both groups were osteoporosis, dyslipidemia, and hypertriglyceridemia. In the HIV- group, three patients were overweight (2 KOA + and 1 KOA-) and we only had one case of arterial hypertension (KOA-) and a patient with prediabetes (KOA +). Regarding the HIV + populations, both groups had similar $CD4^+$ T-cell counts, but the $CD4^+$ Nadir counts found in KOA- patients were lower, yet no statistical significance was found (Table 1).

When evaluating the Kellgren and Lawrence classification, HIV + had a major frequency to suffer from any grade of KOA+, with a relative risk (RR) of 1.9 (95 % CI 0.94–3.7; $P = 0.056$). When evaluating a major risk only for moderate and severe KOA + than mild KOA + in HIV+, we found an RR of 4.0 (95 % CI 0.49–33; $P = 0.075$) without statistical significance (Table 2).

When looking into the serum ghrelin levels, most of the groups had a ghrelin level that reached the maximum detection capacity of the commercial kit used (Table 1). However, the HIV+/KOA + group significantly reduced serum ghrelin levels compared with the other groups ($P = 0.035$) (Figure 1).

Looking into these results, we wanted to identify whether there was a correlation between the ghrelin levels and the HIV + group. Comparing the ghrelin levels with the patients' nadir of $CD4^+$ T-cells count, we identified a statistically significant negative correlation in the KOA- group ($r = -0.80$, $P < 0.007$) (Figure 2).

Due to a significant difference in serum ghrelin concentration identified in HIV+/KOA+, we wanted to evaluate whether the ghrelin levels could be used to identify KOA. An ROC curve analysis was performed to determine the accuracy of ghrelin levels to identified HIV+/KOA + patients from HIV+/KOA- patients. We found an area under the curve (AUC) on 0.83 (95 % CI 0.65–0.10; $P = 0.017$), with a cut-off < 4026 pg/mL serum ghrelin levels, with a sensitivity of 0.62 (95 % CI 0.32–0.86), and a specificity of 0.10 (95 % CI 0.59–0.10) (Figure 3).

4. Discussion

The survival of PLWH has increased thanks to antiretroviral therapy; however, with aging, significant challenges lie ahead, including musculoskeletal complications such as KOA. In addition, it has been reported that PLWH have lower levels

Table 1
Population's demographic and clinical characteristics.

	HIV+/KOA+ (n = 13)	HIV+/KOA- (n = 7)	HIV-/KOA+ (n = 7)	HIV-/KOA- (n = 13)	*P* *
Age (years) mean ± SD	42.6 ± 9	47.0 ± 9	44.7 ± 8	37.7 ± 11	0.332
Gender					
Male	2 (15 %)	1 (14 %)	4 (57 %)	8 (62 %)	
Female	11 (85 %)	6 (86 %)	3 (43 %)	5 (38 %)	
$CD4^{\pm}$ levels (cells/μL) median, IQR	693 (409–873)	663 (450–934)	–	–	0.301
$CD4^{\pm}$ Nadir (cells/μL) median, IQR	238 (155–433)	44 (35–450)	–	_	0.283
BMI (kg/m^2)	25.5 ± 2.4	24 ± 2.2	24 ± 2.7	26 ± 2.6	0.056
Functional Lysholm scale score	81 ± 18	87 ± 17	82 ± 15	94 ± 13	0.096
Kellgren and Lawrence grade					
0	–	1	–	2	
1	–	6	–	11	
2	5	–	6	–	
3	4	–	1	–	
4	–	–	–	–	
Ghrelin serum levels (pg/mL) median, IQR	3075 (2645–7173)	8000 (4112–8000)	8000 (5251–8000)	8000 (3723–8000)	0.035

BMI, body mass index; IQR, interquartile range.
* Kruskal–Wallis test was used.

Table 2
Relative risk (RR) for patients with or without knee osteoarthritis (KOA).

	KOA+	KOA-	
HIV+	13/20 (65 %)	7/20 (35 %)	RR:1.9 (95 %CI 0.94–3.7)
HIV-	7/20 (35 %)	13/20 (65 %)	*P* = 0.056
	Moderate/severe KOA+	Mild KOA+	
HIV+	4/20 (20 %)	16/20 (80 %)	RR:4.0 (95 %CI 0.49–33)
HIV-	1/20 (5 %)	19/20 (95 %)	*P* = 0.075

CI, confidence interval.

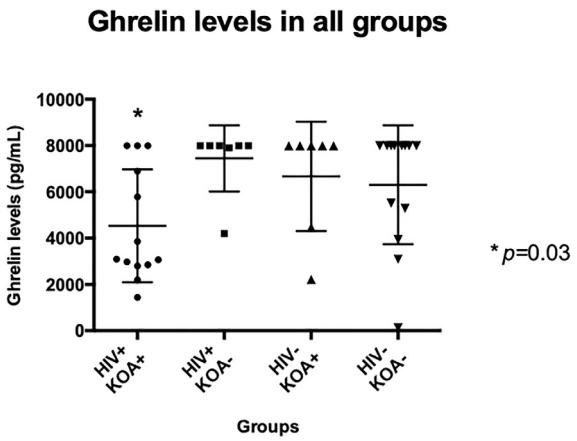

Figure 1. Ghrelin levels in all groups.

of ghrelin compared with healthy controls [15]. Nowadays, ghrelin has been suggested to have a protective role in the pathogenesis and progression of KOA, through different mechanisms [13,14]. Our study aimed to evaluate serum ghrelin levels in HIV + patients with/without KOA and to identify whether there was any association between ghrelin levels and KOA. As far as we can tell, this is the first study to investigate the relationship between the serum levels of ghrelin with radiographic findings and the functional scale of the articulation of the knee in HIV + patients.

In this study, a decrease in ghrelin serum levels was found in the HIV+/KOA + group, compared with HIV+/KOA- patients. Similar information has been reported in patients with an osteoarticular disease without HIV, who had decreased ghrelin levels when there is the presence of cartilage's degenerative features [14]. Following previous reports [15], we were able to detect decreased levels in the HIV+/KOA + group in comparison with non-HIV patients with or without KOA. However, our detection test reached maximum capacity and we were not able to detect decreased ghrelin levels in the HIV+/KOA- group in comparison to the non-HIV groups. It is worth mentioning that, in our study, we have a major presence of females in our study population, mainly in the HIV + groups.

When radiographically assessing the degrees of joint involvement of the knee, our results were similar to the work reported by Y.-C. Zou et al., and patients with grade 2, 3, or 4 KOA had lower ghrelin levels [13]. Our study identified that

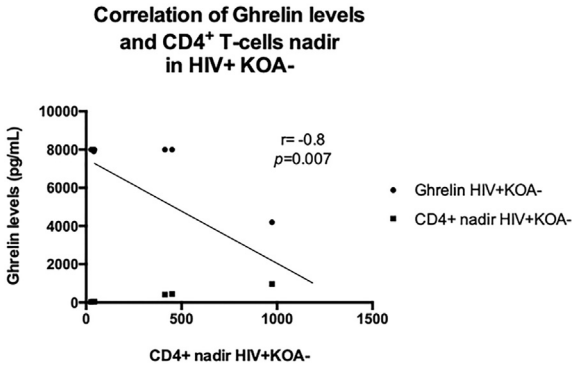

Figure 2. Negative correlation of Nadir of $CD4^+$ T-cells count and ghrelin levels in HIV+/KOA- group. KOA, knee osteoarthritis.

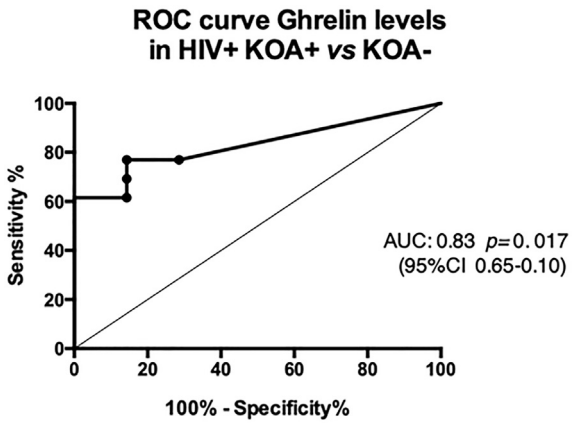

Figure 3. Receiver operating characteristic (ROC) curve analysis of Ghrelin levels in HIV + patients with and without knee osteoarthritis (KOA). AUC, area under the curve.

HIV+/KOA + patients had a more profuse decrease in the ghrelin levels than HIV-/KOA + patients (median concentration per group 3085 vs 8000 pg/mL, $P = 0.036$). However, as we reached the highest detection capacity of our test, we are not capable of detecting differences between the HIV- groups.

Conversely, we found that ghrelin levels were negatively associated with $CD4^+$ T-cells' nadir in HIV + patients. It has been reported that the depletion of $CD4^+$ T-cells disrupts immune homeostasis and promotes chronic immune activation [17]. In accordance with the new findings suggesting ghrelin involvement in inflammation regulation, this negative correlation could suggest a compensation mechanism [9,11–14]. However, due to the ghrelin values reaching the maximum detection capacity of the test, this hypothesis cannot be properly evaluated in this study.

These results further suggest the relevance of ghrelin as a factor in the development of KOA, while also showing the relevance of ghrelin in PLWH, a population not present in other studies and with an intrinsic defect in ghrelin levels. Also, we begin to assess the potential of ghrelin as a new tool for early diagnosis of KOA in PLWH. The performance of the measurement of ghrelin levels considering a cut-off point of < 4026 pg/mL to separate patients who were HIV + with KOA + vs KOA- in the present study resulted in an AUC of 0.83 (95 % CI 0.65–0.10; $P = 0.017$). Similar analysis in the non-HIV population was not possible due to the metrics limitation of the ghrelin levels test. Yet, serum ghrelin measurement could be postulated as a candidate biomarker to search for KOA in HIV + patients. However, due to the transversal nature of this study we were only able to evaluate an association. Thus, longitudinal studies are required to confirm the utility of ghrelin as a biomarker.

It is still necessary to analyze further which ghrelin values can be taken as a diagnostic method in the early stages of KOA to confirm its viability. However, this paper provides an insight regarding ghrelin and KOA in the HIV + populations and provides promising results that encourage follow-up studies of ghrelin as a potential biomarker for PLWH.

5. Conclusion

Our study shows a more focused evaluation of the ghrelin levels and the presence of KOA in a mostly omitted population, the people living with HIV. Also, this study shows initial information of the potential use of ghrelin levels as a biomarker for KOA. However, this work does not come without limitations, such as the sample size and gender bias, and single timepoint sampling. Also, a more sensitive test for measuring ghrelin is required to prevent technical limitations. Hence, further studies are required to confirm our findings. Also, the measure of ghrelin on knee synovial fluid could show the in-site role of ghrelin in KOA, although a blood measurement is more convenient and easier to obtain on a routine basis.

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Author contributions

JIA-R, JFA-V, and LAG-H conceived the project and designed the experiments; JIA-R, GAT-A, JJM-R, and RC-T provided clinical evaluation and follow-up; LAG-H, MA-Z, KS-R, MR-S, and RIC-S performed ghrelin determination and data analysis. LAG-H, JIA-R, and RIC-S and created figures and tables, plus manuscript writing. MA-Z, KS-R, RC-T, and JFA-V reviewed and edited the manuscript. All authors reviewed critically and approved the definitive version of the manuscript to be published.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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