Original Research

Effect of smoking on the redox status of knee osteoarthritis: A preliminary study

Javier Fernández-Torres[1,2*], Octavio Gamaliel Aztatzi-Aguilar[3*], Yessica Zamudio-Cuevas[1], Martha Patricia Sierra-Vargas[4], Gabriela Angélica Martínez-Nava[5], Nathalie Montaño-Armendáriz[1,6], Ambar López-Macay[1], Carlos Suárez-Ahedo[7,8], Victor Ilizaliturri-Sánchez[7], Edicson Jiichiro Nizama-Castillo[7], Anell Olivos-Meza[8], Yazmín Debray-García[4], Alejandra Loaeza-Román[4], Iván Alejandro Luján-Juárez[1,6], Bertha Vargas-Sandoval[9], Roberto Sánchez-Sánchez[10,11], Juana Narváez-Morales[12], Luz María Del Razo[13] and Karina Martínez-Flores[1]

[1]Laboratorio de Líquido Sinovial, Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra," Ciudad de México, C.P. 14389, México; [2]Departamento de Biología, Facultad de Química, Universidad Nacional Autónoma de México (UNAM), Ciudad de México, C.P. 04510, México; [3]Laboratorio de Toxicología de Contaminantes Atmosféricos y Estrés Oxidativo, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Ciudad de México, C.P. 07360, México; [4]Departmento de Investigación en Toxicología y Medicina Ambiental, Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Villegas," Ciudad de México, C.P. 14080, México; [5]Laboratorio de Gerociencias, Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra," Ciudad de México, C.P. 14389, México; [6]Facultad de Medicina, Universidad Autónoma de Guadalajara, Guadalajara, C.P. 45129, México; [7]División de Reconstrucción Articular Cadera y Rodilla, Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra," Ciudad de México, C.P. 14389, México; [8]Departamento de Ortopedia, Hospital Médica Sur, Ciudad de México, C.P. 14040, México; [9]Laboratorio de Microscopía Electrónica, Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra," Ciudad de México, C.P. 14389, México; [10]Unidad de Ingeniería de Tejidos, Terapia Celular y Medicina Regenerativa, Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra," Ciudad de México, C.P. 14389, México; [11]Escuela de Ingenieria y Ciencias, Departamento de Bioingeniería, Tecnológico de Monterrey (TEC), Ciudad de México, C.P. 14380, México; [12]Laboratorio de Toxicología Renal, Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Ciudad de México, C.P. 07360, México; [13]Departamento de Toxicología, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Ciudad de México, C.P. 07360, México

*These authors contributed equally to this paper.

Corresponding author: Karina Martínez-Flores. Emails: karinabiologist@hotmail.com; Kmartinez@inr.gob.mx

Impact Statement

This work is relevant because it characterizes the impact of smoking on patients with osteoarthritis (OA), as it shows that smoking in people under 60 years of age promotes severe OA, probably mediated by an increase in arginase activity, as well as that in adults over 60 years of age. Oxidative stress is promoted by a decrease in antioxidant enzymes. And that the degree of pain is influenced by addiction (smoking), which affects the concentrations of oxidized proteins and the activity of arginase. It has also been shown that smoking 10 or more packs of cigarettes per year increases arginase activity systemically. Arginase activity at the synovial level decreases when cigarette consumption exceeds 10 packs, which is equivalent to 200 cigarettes per year since each pack contains 20 cigarettes. This last finding is of interest because arginase has been linked to the activation of extracellular matrix degrading enzymes such as metalloproteinase.

Abstract

Even though smoking has been scarcely studied in osteoarthritis (OA) etiology, it is considered a controversial risk factor for the disease. Exposure to tobacco smoke has been reported to promote oxidative stress (OS) as part of the damage mechanism. The aim of this study was to assess whether smoking increases cartilage damage through the generation of OS. Peripheral blood (PB) and synovial fluid (SF) samples from patients with OA were analyzed. The samples were stratified according to smoking habit, Kellgren–Lawrence score, pain, and cotinine concentrations in PB. Malondialdehyde (MDA), methylglyoxal (MGO), advanced protein oxidation products (APOPs), and myeloperoxidase (MPO) were assessed; the activity of antioxidant enzymes such as gamma-glutamyl transferase (GGT), glutathione S-transferase (GST) and catalase (CAT), as well as the activity of arginase, which favors the destruction of cartilage, was determined. When stratified by age, for individuals <60 years, the levels of MDA and APOPs and the activity of MPO and GST were higher, as well as antioxidant system activity in the smoking group (OA-S). A greater degree of pain in the OA-S group increased the concentrations of APOPs and arginase activity ($P<0.01$ and $P<0.05$, respectively). Arginase activity increased significantly with a higher degree of pain ($P<0.01$). Active smoking can be an important risk factor for the development of OA by inducing systemic OS in young adults, in addition to reducing antioxidant enzymes in older adults and enhancing the degree of pain and loss of cartilage.

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Introduction

Osteoarthritis (OA) is a chronic disease that causes pain and physical disability. It has been estimated that OA affects 14% of people older than 25 years and almost 34% of people older than 65 years.[1] OA is characterized by the gradual loss of articular cartilage, synovitis, pain, subchondral bone remodeling, and osteophyte formation. It is associated with alterations in amino acids such as arginine, which is metabolized by arginase, an enzyme that catalyzes the hydrolysis of arginine to ornithine and urea, promoting collagen synthesis and cell proliferation, processes involved in fibrosis, a feature associated with OA.[2,3] In addition, arginase activity has been shown to be related to the generation of oxidative stress (OS) because it competes with nitric oxide synthase for arginine to generate nitric oxide, which participates in the catabolism of extracellular matrix (ECM) through the upregulation of metalloproteinases (MMPs).[4,5]

In the early stages, OA is characterized by low-level inflammation, which can occur through the infiltration and activation of macrophages and neutrophils, leading to the production of reactive oxygen species (ROS), which are generated mainly by nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase) and myeloperoxidase (MPO).[6–8] An excess of ROS leads to an inflammatory environment in the joint as a result of an increase in inflammatory mediators (interleukin [IL]-1β, IL-8, IL-17, and tumor necrosis factor α [TNF-α]), which degrade cartilage due to an increase in MMPs and the inhibition of proteoglycan synthesis or type 2 collagen.[9] Changes in joint structure are induced by various factors, such as genetics, sex, obesity, and excessive exercise, and smoking has recently been suggested to be an important risk factor for the development of OA.[8,10]

Tobacco smoke contains more than 5000 harmful toxic chemicals and a series of highly unstable free radicals, which promote the production of ROS and eventually OS.[11] *In vitro* and *in vivo* studies have evaluated the effect that tobacco smoke exerts on the musculoskeletal system. Furthermore, the continuous inhalation of tobacco smoke leads to surgical complications and hospitalization,[12] as well as an increase in total hip arthroplasty failure or complications after total knee arthroplasty.[13,14] Smokers with OA have a higher degree of pain, requiring more prescriptions for narcotics and benzodiazepines to relieve pain after total knee replacement, as well as an increased risk of joint replacement.[15,16] A prospective cohort study indicated that smokers have an increased risk of developing hand OA with a higher degree of pain.[17] In addition, through a combined magnetic resonance analysis, increased damage to the cartilage of the knee joint has been shown to be related to smoking.[8,15] However, studies that have evaluated the relationship between exposure to tobacco smoke and symptomatic OA have limitations; therefore, the mechanisms by which damage is generated in the ECM of the articular cartilage are poorly understood and controversial.[12,17]

It has been suggested that ROS generated by exposure to tobacco smoke increase plasma arginase activity; likewise, in an *in vitro* study of chondrocytes treated with IL-1β, arginase was shown to be overexpressed, a finding related to high levels of arginase and of ECM degradation proteins such as metalloproteinases 3 and 13 (MMP3 and MMP13), suggesting that arginase affects the integrity of cartilage.[5,6,18] The increase in ROS in people with OA and animal models of OA have been identified by the presence of lipoperoxidation products, and through the determination of malonaldehyde (MDA) concentration, in addition to a decrease in antioxidant enzymes that are responsible for neutralizing ROS, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and glutathione (GSH).[6,18]

The objective of this study was to determine whether smoking generates OS as part of the mechanism that promotes the development of OA; this cross-sectional study evaluated plasma and synovial fluid (SF) molecules related to OS status as well as antioxidants. The present work is highly relevant since it will provide evidence of the potential effect of smoking on OS parameters related to the development of OA.

Materials and methods

Description of the work universe

Fifty OA patients treated at the División de Reconstrucción Articular de Cadera y Rodilla del Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra" (INR-LGII) were enrolled. The diagnosis of OA was established using the American College of Rheumatology criteria.[19] Patients were classified radiologically using the Kellgren–Lawrence (KL) score[20] by two independent doctors, and pain intensity was evaluated using a visual analog scale (VAS).

Prior to joint replacement surgery, peripheral blood (PB) samples were collected in vacutainer tubes with EDTA-K_2 anticoagulant, centrifuged to isolate the plasma fraction, and then frozen at −80°C until the biomarkers were measured. SF samples were taken during arthroplasty in 3 mL syringes, subsequently aliquoted into 1.5-mL Eppendorf tubes and frozen at −80°C. Six PB samples could not be processed for the molecular quantifications, and one patient did not meet the minimum volume of SF in order to do all the molecular measurements, so the analysis was carried out with 45 PB samples and 50 SF samples.

This study was conducted in accordance with the criteria of the Declaration of Helsinki,[21] and the protocol (registration code: INR 67/19) was approved by the Ethics and Research Committee of INR-LGII. At the time of recruitment, the participants signed an approved informed consent form.

Smoking parameters

A self-administered questionnaire was used to collect; information on each participant's smoking habits was collected

(smoker/non-smoker, number of cigarettes, and time since initiation) to determine the smoking index (SI). The SI was calculated as follows: number of cigarettes consumed per day divided by the number of years of smoking divided by 20 (number of cigarettes per pack); thus, the number of packs consumed per year was determined, considering a range of greater, less than or equal to 10 (>< or = 10) to determine the effect of smoking on specific parameters.

All patients included in the smoking group (OA-S) were active smokers at the time of enrollment, whereas patients who reported having quit smoking within the last eight years were classified as former smokers (OA-ExS). As a biological marker of tobacco exposure, plasma concentrations of cotinine, a metabolite of nicotine combustion, were determined by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Origene, Cat. EA100901; Rockville, MD, USA).

Quantification of MDA

MDA was quantified by its reaction with *N*-methyl-2-phenylind (MPI); the methodology was based on the procedure described by Esterbauer *et al.*[22] and adapted to a microassay. The reaction product was quantified at 586 nm in a LabSystems Multiskan MS microplate reader (Thermo Fisher Scientific Inc, USA). 1,1,3,3-tetramethoxypropane (10 mM) was used as a standard to generate a standard curve for interpolation of the data obtained.

Quantification of methylglyoxal

Methylglyoxal (MGO) is a product of the oxidation of glucose and lipids and was determined using the 2,4-dinitrophenylhydrazine (DNPH) method adapted to a microassay based on Kwok *et al.*[23] The colored product was read at 540 nm in a microplate reader (LabSystems, Multiskan MS). To quantify MGO, a standard curve was generated using 12.5 mM MGO solution (Sigma-Aldrich, St. Louis, MO, USA), and the results were expressed as mg/total protein mg.

Quantification of advanced protein oxidation products

Advanced protein oxidation products (APOPs) are irreversible protein products containing tyrosine, pentosidine, and carbonyl groups generated during protein oxidation. The quantification of plasma APOPs was measured by spectrophotometry using the method described by Witko-Sarsat *et al.*,[24] based on the exposure of the groups in an acidic medium at a wavelength of 340 nm (Beckman Coulter Du800, Brea, CA, USA). Chloramine-T was used as a standard to generate a standard curve for interpolation of the data. APOPs concentrations were expressed as micromoles of chloramine-T per milligram of total protein (μM chloramine-T/total protein mg).

Evaluation of arginase activity

The method described by Corraliza *et al.*[25] for the quantification of arginase (EC 3.53.1) was adapted to a microtiter plate; the end product of the reaction was read at 540 nm (LabSystems, Multiskan MS). Urea (1 mg/mL) was used as a standard to generate a standard curve. Results were expressed as milligrams of urea per milligram of total protein (mg/total protein mg).

Evaluation of MPO activity

MPO (EC 1.11.2.2) generates hypochlorous acid (HOCl) from hydrogen peroxide and chloride anion. The method described by Suzuki *et al.*[26] was adapted to a microtiter plate format. The MPO reaction product was measured at a wavelength of 590 nm (LabSystems, Multiskan MS). One unit of MPO was defined as a 0.1 change in absorbance. Enzyme activity was expressed as U/total protein mg.

Gamma-glutamyl transferase activity

Gamma-glutamyl transferase (GGT) activity was determined spectrophotometrically at 405 nm in a Lambda 25 UV–Vis Beckman Coulter DU 800 series (Beckman Coulter Inc, USA) through the formation of 5-amino-2-nitrobenzoic acid, which is proportional to the catalytic concentration of GGT in plasma or SF.[27] Enzyme activity was expressed as U/total protein mg.

Glutathione S-transferase activity

Glutathione S-transferase (GST) activity was determined by the method described by Szasz[27] that is, GST catalyzes the nucleophilic attack of the sulfur atom of GSH on the electrophilic groups of the substrate 1-chloro-2,4-dinitrobenzene (DNB). The enzymatic kinetics of GST were quantified at 340 nm using the complex formed by GSH and the substrate (GS-DND) in a Lambda 25 UV–Vis Beckman Coulter DU 800 series. Activity was normalized to total protein concentration. Results are expressed in pmoles/min/total protein mg.

CAT activity

CAT activity in plasma and SF was determined using the method described by Hadwan,[28] modified for a microplate and 50 mmol/L phosphate-buffered saline (PBS) (pH 7.0) for the reaction. Considering that 1 unit of activity corresponds to the loss of 1 μmol H_2O_2 per minute, the molar extinction coefficient ($\varepsilon = 40\ mol^{-1}\ cm^{-1}$) was used to measure the activity.

Protein quantification

Protein concentration was determined using the Bradford assay (Bio-Rad, Hercules, California, USA) and a Lambda 25 UV–Vis Beckman Coulter DU 800 series spectrophotometer. The activity was expressed as U/total protein mg (U/total protein mg). The protein concentration was used to normalize the concentrations of MGO, APOPs, MPO, arginase, CAT, and GGT.

Quantification of OS

Quantification of intracellular ROS in SF samples was performed using the CellROX reagent (Molecular Probes, Life Technologies Cat. C10422®; Carlsbad, CA, USA), which

is designed to measure ROS in living cells. The CellROX reagent emits a strong signal at 640/665 nm as the sample oxidizes. Samples were quantified by image-based cytometry using a Tali Image–Based Cytometer (Invitrogen Life Technologies®). The results were expressed as the percentage of ROS generated, normalized to the number of leukocytes.

Statistical analysis

Results were analyzed using STATA version 14 (Stata Corp., College Station, TX, USA) and GraphPad version 6.01. The difference between the study groups in the distribution of continuous variables was evaluated after stratification by smoking habit, using the Kruskal–Wallis test with adjustment for multiple comparisons using Dunn's test. For categorical variables, absolute and relative frequencies were estimated and compared using Fisher's exact test.

Continuous variables, in addition to estimating medians and interquartile ranges, were categorized based on quartiles to perform stratified analyses by cotinine level, except for the SI variable, which was dichotomized into those with SI <10 and those with SI $\geqslant 10$. Multivariate analysis was also performed to evaluate the association between categorical variables and cotinine levels in PB, as well as a possible interaction between these variables, using multinomial logistic regression. Those variables that showed a significant difference between cases and controls were included in the regression models for adjustment. A 95% confidence level ($\alpha = 0.05$) was set for all analyses; therefore, all P values below 0.05 were considered significant.

Results

Characteristics of the study population

Regarding the self-reported smoking habits of the participants and the diagnosis of OA (21 non-smokers [OA-NS], 17 former smokers [OA-ExS] and 13 smokers [OA-S]), there was a significant difference in the proportion of people under 60 years of age between the OA-S (76.9%) and the OA-NS (14.3%) groups. Regarding gender, the proportion of females was higher in the OA-NS group (80.9%), whereas the proportion of males was 76.9% in the OA-S group ($P < 0.001$).

Regarding the radiologic grade of OA as measured by KL score, we could observe that the OA-NS group had a higher frequency of lower grades than the other groups. While the majority of patients in this group had second- and third-grade OA (42.9% each), the frequency of second- and third-grade OA in the OA-ExS group was 41.2% and 52.9%, respectively, and in the OA-S group, in addition to second- and third-grade OA, 30.8% had fourth-grade OA ($P = 0.29$).

Regarding pain severity, the OA-S and OA-ExS groups had higher pain severity (61.5% and 62.5%, respectively) than the OA-NS group, but the difference was not statistically significant ($P = 0.83$) (Table 1).

Smoking characteristics in the study population

The median SI in the OA-ExS group was three packs/year (interquartile range [IQR] = 5.75) and in the OA-S group was 9.6 packs/year (IQR = 12). This difference was statistically significant ($P = 0.001$). In addition, the OA-S group had a higher cotinine concentration (median = 3.7 ng/mL) than the OA-NS and OA-ExS groups (median = 2.45 and 2.5 ng/mL, respectively) ($P = 0.25$). There were no significant differences in body mass index (BMI) between the groups (Table 2).

Table 1. Characteristics of patients with OA.

Variable	OA non-smokers	OA ex-smokers	OA smokers	*P*
Age (%)				
<60 years	3 (14.29)	1 (5.88)	10 (76.92)	**<0.001**
⩾60 years	18 (85.71)	16 (94.12)	3 (23.08)	
Sex				
Female	17 (80.95)	6 (35.29)	3 (23.08)	**0.001**
Male	4 (19.05)	11 (64.71)	10 (76.92)	
K-L scale (%)				
1	2 (9.52)	0 (0.00)	1 (7.69)	0.293
2	9 (42.86)	7 (41.18)	4 (30.77)	
3	9 (42.86)	9 (52.94)	4 (30.77)	
4	1 (4.76)	1 (5.88)	4 (30.77)	
VAS (%)				
Mild	0 (0.00)	1 (6.25)	1 (7.69)	0.829
Moderate	4 (21.05)	1 (6.25)	1 (7.69)	
Severe	4 (21.05)	4 (25.00)	3 (23.08)	
Unbearable	11 (57.89)	10 (62.50)	8 (61.54)	

OA: osteoarthritis; K-L: Kellgren–Lawrence; VAS: visual analog scale.
Variables were expressed as frequency (%).
The *P* value was estimated with Fisher's exact test, $P < 0.05$; $P < 0.001$.
$P < 0.001$ bold significant difference in the proportion of people under 60 years of age between the OA-S and OA-NS groups. $P = 0.001$ bold significant difference in the proportion of women in the OA-NS group compared to men in the OA-S group.

Comparison of the redox state

Plasma levels of MDA were slightly elevated in the OA-S group compared to the OA-NS and OA-ExS groups, but the difference was not statistically significant ($P = 0.52$). Regarding plasma APOPs, the levels tended to be higher in the OA-S group (0.43 µM chloramine-T/total protein mg) than in the OA-NS group (0.32 µM chloramine-T/total protein mg) and the OA-ExS group (0.34 µM chloramine-T/total protein mg), but the difference was not significant ($P = 0.39$). Plasma GGT activity was significantly higher in the OA-S group (1.31 U/total protein mg) than in the OA-NS group (1.04 U/total protein mg) and the OA-ExS group (0.93 U/total protein mg) ($P = 0.06$).

There were no significant changes in the antioxidant system. Plasma GST activity was higher in the OA-S group (0.56 pmoles/min/total protein mg) than in the OA-NS and OA-ExS groups (both with values of 0.53 pmoles/min/total protein mg) ($P = 0.79$). In contrast, CAT activity was lower in the OA-S group than in the other groups.

Regarding SF, OS was slightly higher in the OA-S group (0.115%) than in the OA-NS and OA-ExS groups (both 0.07) ($P = 0.84$). Concentrations of MDA (0.75 µmoles) and MGO (11.39 mg/total protein mg) were slightly higher in the OA-S group than in the OA-NS (0.70 µmoles; 7.79 mg/total protein mg) and OA-ExS (0.68 µmoles; 8.49 mg/total protein mg) groups ($P > 0.71$). GGT activity tended to be higher in the OA-S group than in the OA-NS and OA-ExS groups ($P > 0.99$). CAT activity was slightly higher in the OA-S group (0.007 U/total protein mg) than in the OA-NS and OA-ExS groups ($P = 0.19$). Finally, arginase activity was

Table 2. Characteristics of smoking in the population studied.

Variable	OA non-smokers	OA ex-smokers	OA smokers	*P*
Plasma				
Cotinine (ng/mL)	2.45 (0.899)	2.5 (1.2)	3.7 (11.7)	0.2464
Smoking index (pack years)	0 (0)	3 (5.75)[a]	9.6 (12)[a]	**0.0001**
Age (years)	68 (9)	63 (7)	57 (6)[ab]	**0.0017**
BMI (kg/m^2)	28.89 (5.20)	28.6 (2.57)	27.73 (10.21)	0.6803

OA: osteoarthritis; BMI: body mass index.
The variables were expressed in medians (IQR).
[a]Significant *P* value with respect to group non-smokers.
[b]Significant *P* value with respect to the group of ex-smokers.
Dunn's multiple comparison test was used with $P < 0.05$; $P \leq 0.0001$. $P = 0.0001$ bold significant difference in median smoking index between OA-ExS and OA-S groups with respect to OA-NS. $P = 0.0017$ bold significant difference in the age of the OA-S group with respect to OA-NS and OA-ExS.

lower in the OA-S group than in the OA-NS and OA-ExS groups ($P = 0.96$) (Table 3).

Redox state in smokers with OA stratified by age

Regarding plasma OS parameters in patients under 60 years of age, the concentration of MDA was higher in the OA-S group (0.70 µmoles) than in the OA-NS (0.62 µmoles) and OA-ExS (0.68 µmoles) groups, as were the levels of APOPs. GST activity in the OA-S group was 0.58 pmoles/min/total protein mg, which was higher than in the OA-NS (0.44 pmoles/min/total protein mg) and OA-ExS (0.39 pmoles/min/total protein mg) groups ($P = 0.05$). In SF, the concentration of MDA was slightly lower in the OA-S group (0.75 µmoles) than in the OA-ExS group (0.82 µmoles), but not significantly different.

MPO activity was slightly higher in the OA-S group (1.100 U/total protein mg) than in the OA-NS group (0.55 U/total protein mg) and the OA-ExS group (0.23 U/total protein mg) ($P = 0.12$). Arginase activity was higher in the OA-S group (3.39 mg urea/total protein mg) than in the OA-NS and OA-ExS groups (1.22 and 1.09 mg urea/total protein mg, respectively) ($P = 0.12$) (Table 4).

Plasma MDA concentration in patients older than 60 years was higher in the OA-S group (0.96 µmoles) than in the OA-NS group (0.80 µmoles) and the OA-ExS group (0.64 µmoles). GGT activity was significantly higher in the OA-S group (1.47 U/total protein mg) than in the OA-NS and OA-ExS groups (1.00 and 0.94 U/total protein mg, respectively) ($P = 0.04$). GST activity was significantly lower in the OA-S group (0.42 pmoles/min/total protein mg) than in the OA-NS and OA-ExS groups (0.52 and 0.54 pmoles/min/total protein mg, respectively) ($P = 0.53$).

In SF, OS tended to be higher in the OA-S group (0.12%) than in the OA-NS (0.09%) and OA-ExS (0.07%) groups. MGO was lower in the OA-S group (0.21 mg/total protein mg) than in the OA-NS (0.37 mg/total protein mg) and OA-ExS (0.32 mg/total protein mg) groups ($P = 0.35$). MPO was lower in the OA-S group (0.79 U/total protein mg) than in the OA-NS (1.67 U/total protein mg) and OA-ExS (1.88 U/total protein mg) groups ($P = 0.10$). GGT was lower in the OA-S group (0.14 U/total protein mg) than in the OA-NS group (0.19 U/total protein mg) and OA-ExS group (0.15 U/total protein mg) ($P = 0.90$). CAT activity was lower in the OA-S group (0.005 U/total protein mg) than in the OA-NS (0.02 U/total protein mg) and OA-ExS (0.03 U/total protein mg) groups ($P = 0.10$), and arginase activity was lower in the OA-S group (3.09 mg urea/total protein mg) than in the OA-NS (3.65 mg) and OA-ExS (3.89 mg/total protein mg) groups (Table 5).

In the bivariate analysis of SF concentrations of MGO and age quartiles, the concentration significantly decreased in Q2 (61–68 years) compared to Q1 (40–60 years) ($P = 0.007$) (Figure 1(a)). However, when stratified by smoking status, MGO concentration significantly increased in the Q2 OA-S group compared to the Q1 OA-S group ($P = 0.03$) and also significantly increased in the Q2 OA-NS group compared to the Q1 OA-S group ($P = 0.035$) (Figure 1(b)).

Effect of cotinine on MGO concentrations and arginase and CAT activity at the plasma level

In the multivariate analysis, at high cotinine concentrations (3.1–67.6 ng/mL), there was a 12.5-time less possibility of having a medium MGO concentration (odds ratio [OR] = 0.08, $P = 0.02$) and a 10-time less possibility of having a high MGO concentration (OR = 0.10, $P = 0.03$), when adjusted for age, BMI, and plasma arginase activity (Table 6).

When multivariate analysis was performed using categorized levels of arginase in PB, there was a marginal positive OR between average cotinine concentration and high levels of arginase, suggesting that at average levels of cotinine, there is a greater likelihood of having higher plasma arginase activity (OR = 14.38, $P = 0.05$), adjusting for age and BMI. With moderate cotinine levels, there is a greater likelihood of having moderate plasma CAT activity (OR = 12.60, $P = 0.04$), adjusted for BMI (Table 6). In addition, there was a significant interaction between cotinine concentration and arginase activity ($P = 0.01$) on plasma MGO levels when adjusted for BMI and age. There was also a marginal interaction between cotinine concentration and BMI ($P = 0.057$).

MPO activity by age in SF and plasma arginase activity by smoking status

Plasma MPO activity decreased with higher arginase activity in the OA-S group compared to the OA-NS group; however, a significant increase was observed when compared by arginase quartiles. MPO activity was higher in the third quartile of arginase in the OA-S group than in the first quartile in the OA-NS and OA-S groups ($P = 0.007$ and $P = 0.008$, respectively). This higher activity of MPO was also observed in

Table 3. Oxidative stress among patients with OA and smoking.

Variable	OA non-smokers	OA ex-smokers	OA smokers	*P*
Plasma				
MDA (µmoles)	0.77 (0.505)	0.65 (0.215)	0.88 (0.600)	0.5170
MGO (mg/total protein mg)	4.43×10^{4} (3.18×10^{-4})	4.67×10^{-4} (3.57×10^{-4})	4.42×10^{-4} (1.08×10^{-4})	0.3882
APOPs (µM cloramine-T/total protein mg)	0.32 (0.147)	0.34 (0.122)	0.43 (0.173)	0.3906
MPO (U/total protein mg)	5.46×10^{-2} (3.14×10^{-2})	6.00×10^{-2} (1.92×10^{-2})	5.35×10^{-2} (4.65×10^{-2})	0.8383
GGT (U/total protein mg)	1.04 (0.224)	0.93 (0.458)	1.31 (0.360)	0.0607
GST (pmoles/min/total protein mg)	0.53 (9.21×10^{-2})	0.53 (0.165)	0.56 (0.129)	0.7861
Catalase (U/total protein mg)	3.24×10^{-3} (4.71×10^{-3})	4.45×10^{-3} (3.87×10^{-3})	2.26×10^{-3} (6.64×10^{-3})	0.5323
Arginase (mg urea/total protein mg)	4.19×10^{-4} (1.13×10^{-4})	4.64×10^{-4} (1.35×10^{-4})	4.37×10^{-4} (7.49×10^{-5})	0.3086
Sinovial fluid				
Oxidative stress (OS) (%)	0.07 (0.12)	0.07 (0.13)	0.115 (0.15)	0.8431
MDA (µmoles)	0.697 (0.299)	0.676 (0.2995)	0.748 (0.496)	0.7404
MGO (mg/total protein mg)	7.794 (13.789)	8.493 (10.0915)	11.391 (11.191)	0.7157
APOPs (µM cloramine-T/total protein mg)	0.1905 (0.421)	0.158 (0.343)	0.198 (0.147)	0.9989
MPO (U/total protein mg)	1.54 (1.525)	1.582 (1.8455)	1.049 (0.591)	0.4891
GGT (U/total protein mg)	0.1905 (0.421)	0.158 (0.343)	0.198 (0.147)	0.9989
GST (pmoles/min/total protein mg)	3.278 (10.568)	3.263 (3.194)	2.374 (2.09)	0.5119
Catalase (U/total protein mg)	0.011 (0.067)	0.026 (0.0335)	0.007 (0.018)	0.1936
Arginase (mg urea/total protein mg)	3.565 (10.46)	3.7555 (2.95)	3.289 (2.272)	0.9614

OA: osteoarthritis; MGO: methylglyoxal; MDA: malodhyaldehyde; APOPs: advanced oxidation products; MPO: myeloperoxidase; GST: glutathione S-transferase; GGT: gamma-glutamyl transferase.
The variables were expressed in medians (IQR).
Dunn's multiple comparison test was used with $P \leqslant 0.05$; $P \leqslant 0.0001$.

Table 4. Oxidative stress parameters in smoking patients under 60 years of age.

Variable	OA non-smokers	OA ex-smokers	OA smokers	*P*
Plasma				
Smoking index (packs years)	0 (0)	0.085 (0)	9.05 (12.3)[ab]	**0.0162**
MDA (µmoles)	0.62 (0.36)	0.68 (0)	0.71 (0.9)	0.8981
MGO (mg/total protein mg)	4.63×10^{-4} (1.35×10^{-4})	6.34×10^{-4} (0)	4.52×10^{-4} (1.43×10^{-4})	0.2293
APOPs (µM chloramine-T/total protein mg)	0.32 (0.082)	0.33 (0)	0.45 (0.172)	0.3269
MPO (U/total protein mg)	6.16×10^{-2} (4.60×10^{-2})	3.95×10^{-2} (0)	7.32×10^{-2} (4.86×10^{-2})	0.4702
GGT (U/total protein mg)	1.40 (5.640)	0.66 (0)	1.07 (0.323)	0.2293
GST (pmoles/min/total protein mg)	0.44 (0.106)	0.39 (0)	0.58 (0.072)	0.0512
Catalase (U/total protein mg)	4.23×10^{-3} (4.02×10^{-3})	4.72×10^{-3} (0)	4.73×10^{-3} (5.88×10^{-3})	0.8491
Arginase (mg urea/ total protein mg)	4.70×10^{-4} (2.02×10^{-4})	4.40×10^{-4} (0)	4.63×10^{-4} (1.56×10^{-4})	0.9131
Sinovial fluid				
Oxidative stress (OS) (%)	0.03 (0.02)	0.21 (0)	–	0.2207
MDA (µmoles)	0.75 (0.753)	0.82 (0)	0.75 (0.568)	0.8937
MGO (mg/total protein mg)	0.16 (0.243)	0.17 (0)	0.34 (0.363)	0.2975
MPO (U/total protein mg)	0.55 (1.873)	0.23 (0)	1.10 (1.291)	0.1234
GGT (U/total protein mg)	0.18 (0.143)	0.38 (0)	0.23 (0.165)	0.3129
GST (pmoles/min/total protein mg)	1.70 (3.518)	1.07 (0)	2.75 (2.367)	0.2654
Catalase (U/total protein mg)	0.006 (0.11)	0.11 (0)	0.02 (0.24)	0.3954
Arginase (mg urea/total protein mg)	1.22 (2.66)	1.09 (0)	3.39 (3.641)	0.1209

OA: osteoarthritis; MGO: methylglyoxal; MDA: malodhyaldehyde; APOPs: advanced oxidation products; MPO: myeloperoxidase; GST: glutathione S-transferase; GGT: gamma-glutamyl transferase.
The variables were expressed in medians (IQR).
[a]Significant *P* value with respect to the group of non-smokers.
[b]Significant *P* value with respect to the group of ex-smokers.
Dunn's multiple comparison test was used with a value of $P \leqslant 0.05$. $P = 0.0162$ bold significant difference in the smoking index of OA-S under 60 years of age with respect to OA-NS and OA-ExS.

the third quartile of arginase in the OA-NS group compared to the first quartile in the OA-NS and OA-S groups ($P = 0.02$ and $P = 0.03$, respectively) (Figure 2(a)).

In SF, MPO activity when stratified by age quartiles significantly increased in Q2 compared to Q1 ($P = 0.003$) (Figure 2(b)). Furthermore, MPO activity showed significant differences when stratified by age and smoking status. The highest MPO activity was observed in the second quartile of age in the OA-NS group, followed by that observed in the OA-S group for the same quartile of age. This difference

Table 5. Oxidative stress parameters in patients older than 60 years according to their smoking index.

Variable	OA non-smokers	OA ex-smokers	OA smokers	*P*
Smoking index (pack years)	0 (0)	3.25 (7.75)[a]	9.6 (34.5)[a]	**0.0001**
Plasma				
MDA (μmoles)	0.80 (0.54)	0.64 (0.25)	0.96 (0.65)	0.2802
MGO (mg/total protein mg)	4.23×10^{-4} (3.33×10^{-4})	4.63×10^{-4} (4.40×10^{-4})	3.66×10^{-4} (2.47×10^{-4})	0.3286
APOPs (μM chloramine-T/total protein mg)	0.32 (0.1450)	0.34 (0.1310)	0.39 (0.3643)	0.7171
MPO (U/ total protein mg)	4.89×10^{-2} (2.87×10^{-2})	6.03×10^{-2} (2.15×10^{-2})	5.05×10^{-2} (2.83×10^{-2})	0.5461
GGT (U/total protein mg)	1.00 (0.1426)	0.94 (0.4518)	1.47 (0.4046)[ab]	**0.0414**
GST (pmoles/min/total protein mg)	0.52 (0.0830)	0.53 (0.1694)	0.42 (0.2508)	0.5329
Catalase (U/total protein mg)	3.10×10^{-3} (4.86×10^{-3})	4.17×10^{-3} (4.26×10^{-3})	5.66×10^{-4} (4.11×10^{-3})	0.2896
Arginase (mg urea/total protein mg)	3.95×10^{-4} (9.55×10^{-5})	4.65×10^{-4} (1.54×10^{-4})	4.32×10^{-4} (5.63×10^{-5})	0.2690
Sinovial fluid				
Oxidative stress (OS)(%)	0.09 (0.15)	0.07 (0.09)	0.12 (0.15)	0.8295
MDA (μmoles)	0.69 (0.35)	0.64 (0.31)	0.65 (1.05)	0.8369
MGO (mg/total protein mg)	0.37 (1.241)	0.32 (0.239)	0.21 (0.296)	0.35
MPO (U/total protein mg)	1.67 (1.40)	1.88 (2.01)	0.79 (0.47)	0.10
GGT (U/total protein mg)	0.19 (0.44)	0.15 (0.34)	0.14 (0.07)	0.90
GST (pmoles/min/total protein mg)	3.74 (16.70)	3.44 (3.18)	1.96 (1.31)	0.22
Catalase (U/total protein mg)	0.02 (0.233)	0.03 (0.41)	0.005 (0.001)	0.10
Arginase (mg urea/total protein mg)	3.65 (13.51)	3.89 (13.51)	3.09 (2.98)	0.57

OA: osteoarthritis; MGO: methylglyoxal; MDA: malodhyaldehyde; APOPs: advanced oxidation products; MPO: myeloperoxidase; GST: glutathione S-transferase; GGT: gamma-glutamyl transferase.
The variables were expressed in medians (IQR).
[a]Significant *P* value with respect to the group of non-smokers.
[b]Significant *P* value with respect to the group of ex-smokers.
Dunn's multiple comparison test was used with $P \leqslant 0.05$; $P \leqslant 0.0001$. $P = 0.0001$ bold significant difference in the smoking index of OA-S and OA-ExS older than 60 years with respect to OA-NS. $P = 0.0414$ bold significant difference in GGT activity of OA-S with respect to OA-NS and OA-ExS.

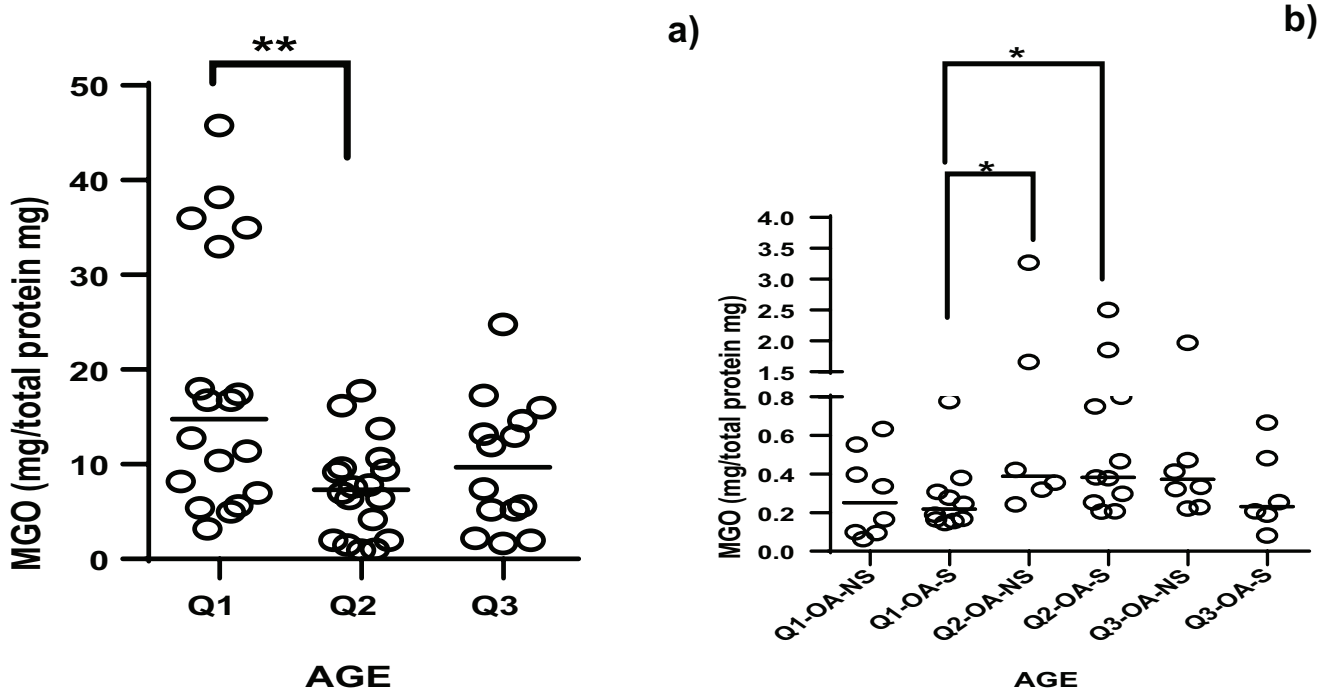

Figure 1. Relationship of age with respect to MGO. (a) The higher the age, the lower the MGO concentration (**$P = 0.007$). (b) OA smokers (OA-S) and non-smokers (OA-NS) (both quartile 2) presented significant increases (*$P = 0.03$ and *$P = 0.035$) in MGO concentration with respect to the age quartile 1 of the OA smokers (OA-S).

was significant when compared to its activity in the first quartile of age in the OA-NS and OA-S groups ($P = 0.03$ and $P = 0.01$, respectively), in addition to its difference with respect to MPO activity in the first quartile of age in the OA-S group ($P = 0.02$) (Figure 2(c)).

Levels of APOPs and arginase activity on smokers with OA with different levels of pain

The production of APOPs increased significantly in the OA-S group compared to the OA-NS group when comparing only

Table 6. Multivariate analysis of the effect of cotinine on enzymes involved in developing osteoarthritis.

	Cotinine concentration level					
	Medium			High		
	OR	*P*	95% CI	OR	*P*	95% CI
MGO plasma concentration level						
Medium	1.68[a]	0.70	0.12–23.70	**0.08**[a]	**0.02**	**0.01–0.63**
High	1.68[a]	0.70	0.12–24.52	**0.10**[a]	**0.03**	**0.87–1.08**
Arginase plasma activity level						
Medium	0.80[b]	0.87	0.05–13.47	0.29[b]	0.19	0.04–1.82
High	14.38[b]	0.05	0.96–214.61	1.38[b]	0.73	0.22–8.75
Catalase plasma activity level						
Medium	**12.60**[c]	**0.04**	**1.06–149.51**	1.59[c]	0.64	0.22–11.54
High	4.36[c]	0.29	0.28–68.45	3.92[c]	0.16	0.59–26.09

OR: odds ratio; CI: confidence interval; MGO: methylglyoxal.
Text in bold denotes statistical significance.
[a]Adjusted by age, BMI, and arginase plasma concentration.
[b]Adjusted by age and BMI.
[c]Adjusted by BMI.

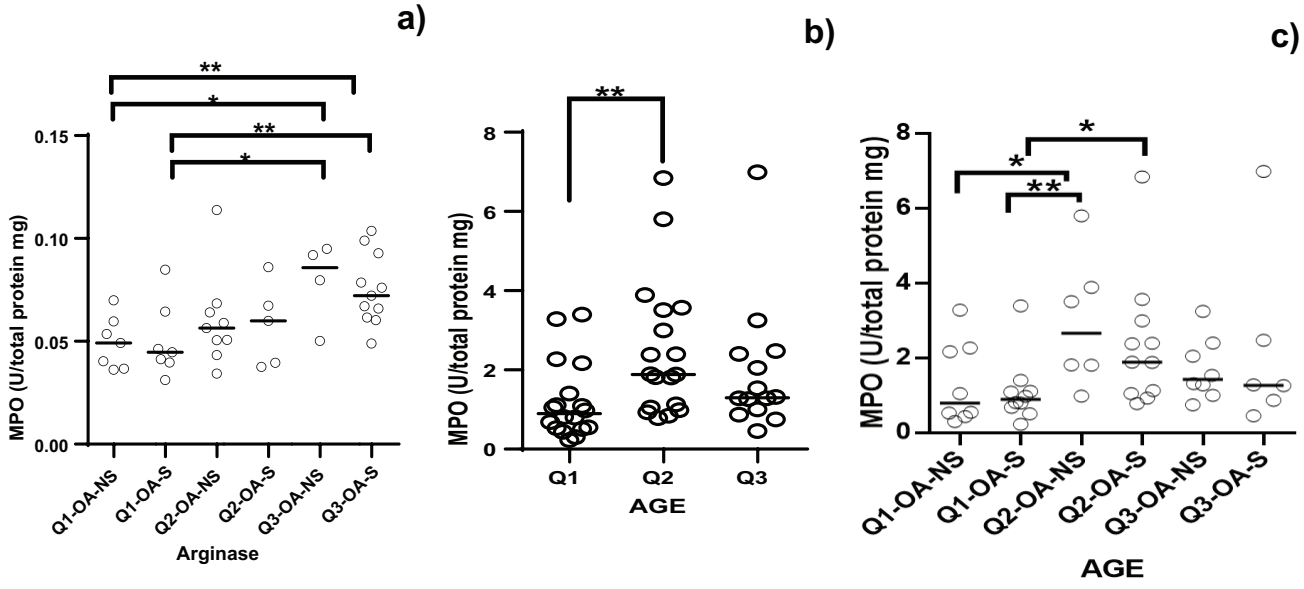

Figure 2. Effect on MPO activity with respect to arginase activity and age. (a) OA-S had higher MPO Q3 compared to OA-NS and OA-S groups to MPO Q1 (**P = 0.007 and **P = 0.008). (b) MPO was significantly higher in Q2 when consider age than Q1 (**P = 0.0034). (c) MPO Q2 was significantly higher in the OA-NS and OA-S groups when consider age than MPO Q1 in the OA-S group when considering age (*P = 0.0280 and *P = 0.0243).

the patients with the lowest level of pain (VAS Q1) (P = 0.003). The OA-S group with the highest degree of pain (VAS Q3) had significantly higher APOPs production than the OA-NS group with a lower degree of pain (VAS Q1) (P = 0.03). In addition, APOPs production was higher in the OA-S group with higher pain (VAS Q3) than in the OA-NS group with moderate pain (VAS Q2) (P = 0.04) (Figure 3(a)).

Regarding arginase activity, for VAS Q1, arginase activity was higher in the OA-S group than in the OA-NS group (P = 0.05). For VAS Q3, arginase activity was higher in the OA-S group than in the OA-NS group (P = 0.02). In addition, there was a significant difference in arginase activity between OA-S groups belonging to VAS Q2 and Q3 (P = 0.01) (Figure 3(b)).

Arginase activity in smokers with OA

Plasma arginase activity increased significantly (P = 0.04) with a smoking rate of more than 10 packs per year (Figure 4(a)). In relation to pain severity, arginase activity was significantly higher in Q3 than in Q2 (P = 0.006) (Figure 4(b)). GST activity changed in relation to arginase activity and self-reported smoking; it was significantly higher in the OA-S group with the highest arginase activity (Q3) than in the OA-S group with intermediate arginase activity (Q2), as well as in the OA-NS group with the lowest arginase activity (Q1) (P = 0.01 and P = 0.007, respectively) (Figure 4(c)). In SF, arginase activity was lower when SI was greater than 10 (P = 0.55) (Figure 4(d)).

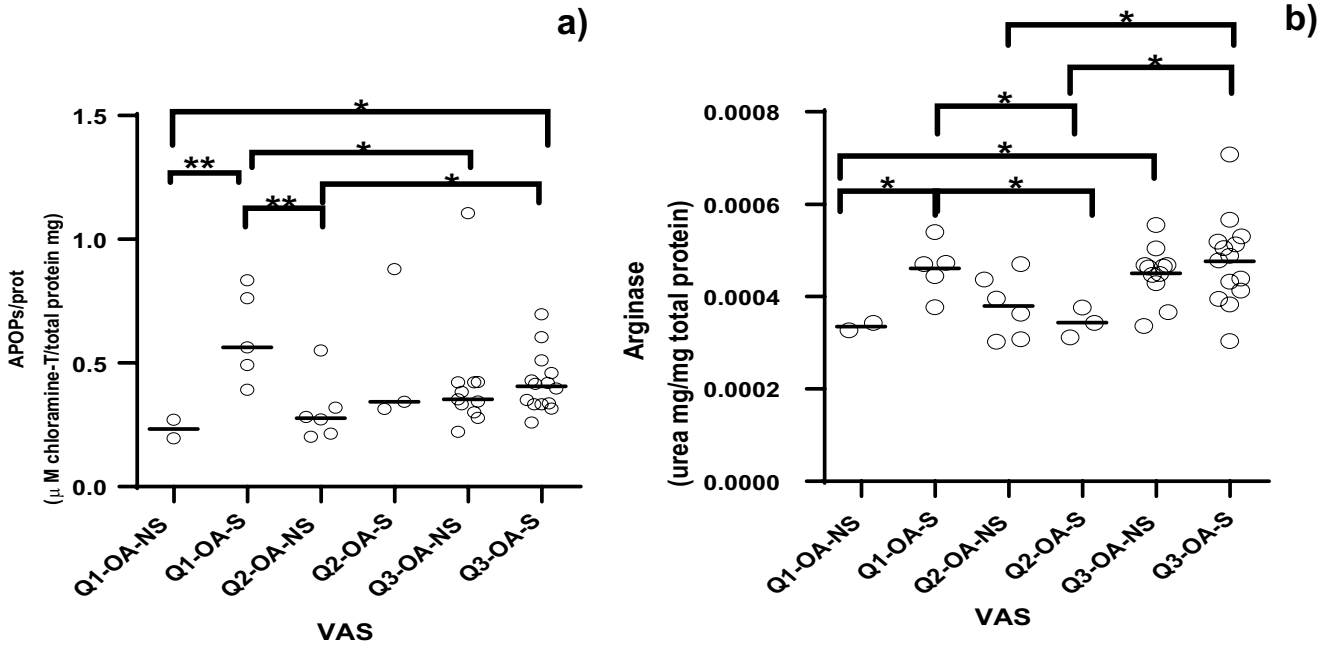

Figure 3. Impact of the degree of pain on the levels of APOPs and arginase activity. (a) Smokers group had the highest degree of pain (VAS Q3) had significantly higher APOP production than did the OA-NS group with a lower degree of pain (VAS Q1 and Q2) (*$P = 0.0289$ and *$P = 0.0409$). Comparing the group with the lowest pain level Q1, there was a significant incrase in APOPs in OA-S compared to OA-NS and also compared to the group of OA-NS with the highest pain level Q2 (**$P = 0.0029$ and **$P = 0.0023$). (b) For VAS Q1 and Q2, arginase activity was greater in the OA-S group than in the OA-NS group (*$P = 0.0499$ and *$P = 0.0184$).

Discussion

Smoking has been associated with low back pain and degenerative disk disease. A study by Hoxha *et al.*[29] reported a higher percentage of male smokers with OA (37.2%) than female smokers with OA (13.1%). In a 2013 study, patients who smoked had radiographic evidence of OA greater than 2 on the KL scale.[30] These findings support our results that there was a higher percentage of grade 4 OA in smokers (30.8%) than in non-smokers (4.8%) and ex-smokers (5.9%), suggesting that smoking is a factor that contributes to greater cartilage loss in smokers than in those who do not smoke or who used to smoke but have quit. In addition, Bartolone *et al.*[31] reported that men had the highest proportion of smokers with OA (54.55%), while women had 36.11%. These findings are similar to our study population, in which 76.92% were male smokers with OA and 23.08% were female smokers with OA. Of note, the proportion of males to females in the non-smoking OA group was almost reversed, that is, 80.95% females and 19.05% males, suggesting that smoking is more conducive to the development of OA in males, although OA is considered to be a disease that predominantly affects females, as pointed out by Zhang *et al.*,[32] who reported that worldwide, 18% of females develop OA compared to only 10% of males. One hypothesis is that smoking promotes inflammatory processes leading to cartilage loss.

In addition, Oliver and Silman[33] suggest that smoking intensity, duration, and time since quitting interact with genetic factors that predispose to the development of musculoskeletal disease. Smoking has also been associated with the progression of symptomatic knee OA. Regarding pain, according to Shi *et al.*,[34] smoking patients tend to report more severe joint pain than non-smoking patients; however, our results did not show a significant difference in the percentage of smoking and non-smoking patients with OA manifested by intractable pain. Regarding pain, Dubé *et al.*[35] reported that patients with OA who actively smoked had more pain than those who had never smoked. In addition, Amin *et al.*[8] reported that smokers had higher VAS scores compared to non-smokers both at baseline and during follow-up. However, in our study, there were no significant differences in pain among non-smokers, former smokers, and smokers, probably because of the limited number of participants; however, a trend was observed in the degree of excruciating pain reported by former smokers and smokers. Among smokers, there was also a significant relationship between higher levels of pain and higher concentrations of APOPs and arginase activity. According to Pascale *et al.*,[36] arginase activity is increased in inflammatory processes. In addition, we found that patients with OA who were smokers were younger than non-smokers and former smokers. BMI was lower in smokers than in non-smokers and former smokers. These findings were similar to those reported in a Danish population, in which patients with OA who were smokers were younger than non-smokers, and the former had a lower BMI.[37]

OS is part of the physiopathogenic mechanism of OA. Tobacco smoke is a source of superoxide anion (O2.–) and hydroxyl (OH.–) and peroxyl (ROO) radicals, which are capable of initiating lipoperoxidation processes. In this sense, we evaluated the levels of MDA, as a final product of lipoperoxidation, and found higher plasma MDA concentrations in the group of smokers with OA than in non-smokers or ex-smokers with OA, as well as a significantly higher concentration of SF in the OA-S group than in the other groups. When stratified by age, those under 60 years of age had high plasma MDA concentrations, especially in the OA-S group; however, SF concentrations were higher in

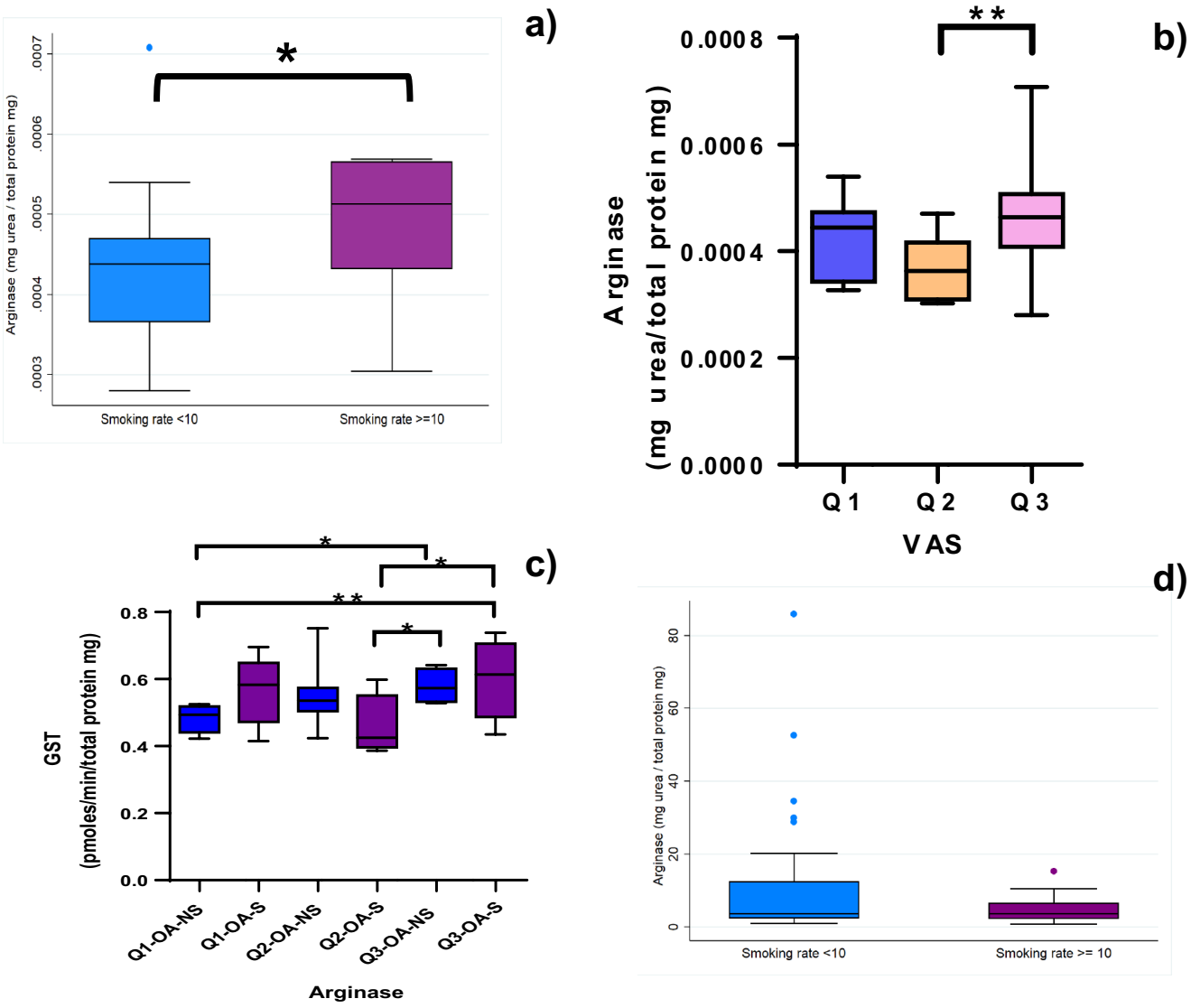

Figure 4. Arginase activity modulated by smoking and degree of pain. (a) Consumption of more than 10 packs per year significantly increases plasma arginase activity (*$P = 0.04$). (b) Arginase activity was significantly higher for Q3 than for Q2 (**$P = 0.0062$). (c) The higher the arginase activity (Q3) in OA-S the lower the GST activity in Q1 OA-NS as well as in Q2 OA-S (**$P = 0.0074$ and *$P = 0.0101$). OA-NS with higher arginase activity (Q3) have lower GST activity compared to OA-NS Q1 and OA-S Q2 (*$P = 0.0398$ and *$P = 0.0394$). (d) Arginase activity in synovial fluid by smoking index ($P = 0.55$).

former smokers, suggesting that smoking increases cartilage damage. In the group over 60 years of age, plasma MDA concentrations were higher in the OA-S group than in the OA-NS and OA-ExS groups; however, there were no differences between the groups for MDA in the SF.

These findings are relevant because MDA mediates the oxidation of collagen in cartilage,[38] and smoking tends to increase MDA levels. In addition, levels of MGO – a highly reactive metabolite formed during glucose, protein, and fatty acid metabolism – are associated with the production of OS and are particularly elevated in patients diagnosed with OA and disk degeneration associated with diabetes.[32,39,40] These results contrast with the findings in our study population, that is, at the plasma level, a lower concentration of MGO was observed in the smoker group than in non-smokers and former smokers. We also found that there was a higher concentration of cotinine and a lower concentration of MGO, and when the population was stratified by MGO concentration and tobacco dependence, smokers had a higher concentration of cotinine than non-smokers. One explanation for these results is that patients with co-morbidities such as diabetes were excluded to reduce the influence of these factors on the development and severity of OA, thus allowing us to highlight the effect of smoking at the joint level. For MGO in SF, when stratified by age, Q2 was significantly lower than Q1. However, when stratified by age and tobacco dependence, Q2 increased significantly in both OA-NS and OA-S compared to OA-S in Q1. This finding is particularly important in OA patients who smoke, as tobacco smoke is an exogenous source of advanced glycation products, of which MGO is a precursor.[39]

Other oxidative molecules associated with proteoglycan damage are APOPs,[41] and plasma APOPs were higher in smokers than in the other groups. When grouped by age, APOPs were higher in the OA-S group than in the other groups, both below and above 60 years of age, suggesting

that smoking favors the generation of these oxidation products, which are considered biomarkers of OS. A study by Liao *et al.*[41] suggests that APOPs are positively correlated with the degree of OA because they promote cartilage damage by inducing chondrocyte apoptosis and increasing MMP-13 expression. However, more research is needed to strengthen these findings, as there are no studies linking smoking as an enhancer of APOPs in the development of OA.

MPO activity, in plasma and SF, was lower in smokers than in non-smokers and former smokers. When considering those under 60 years of age, MPO activity was higher in the plasma and SF of participants in the OA-S group than in the OA-NS and OA-ExS groups. When considering people older than 60 years, plasma MPO activity was higher in the OA-S and OA-ExS groups than in the OA-NS group, and SF MPO activity was lower in the OA-S group than in the OA-NS and OA-ExS groups. These findings were supported by subsequent analyses: lower MPO activity in smokers who presented higher arginase activity and lower MPO activity in older smokers. Steinbeck *et al.*[7] suggested that MPO activity is elevated in SF in the early stages of OA. In our study, we found greater MPO activity not only in SF but also in plasma in smokers under and over 60 years of age; however, in the SF of patients older than 60 years, MPO activity decreased. In addition, in the bivariate analysis, it reinforced the previous results, demonstrating that age by itself tends to increase MPO activity; however, smoking decreases it in ages over 60 years, in contrast to those under 60 years. These findings could be explained since smoking has been shown to increase the activity of the enzyme, which is involved in the generation of ROS, which favor the process of cartilage loss.[42,43] In addition, no studies have evaluated the effect of tobacco on MPO activity in patients with OA; therefore, our results are the first to suggest that smoking favors greater MPO activity, possibly through the activation of phagocytic cells, especially polymorphonuclear (PMN) cells, enhancing joint damage because MPO in the presence of chlorides is capable of generating HOCL and chlorine molecules, which are highly reactive and capable of oxidizing type II collagen, one of the main ECM proteins, increasing the susceptibility of type II collagen to proteolytic degradation.[7]

In this study, ROS was only assessed in SF because the amount of plasma sample obtained was insufficient to perform all assays. There was a higher OS in the OA-S group than in the OA-NS and OA-ExS groups; however, when compared by age, ex-smokers had a higher OS than never-smokers. In those over 60 years of age, the increase in OS was maintained in the OA-S group; that is, age favors ROS generation, but smoking intensity, which has a greater impact on OA, generates a wide variety of ROS that can accumulate in the joints and promote the progression of OA.[44]

To counteract OS, antioxidant molecules are activated, such as CAT, which converts hydrogen peroxide to water and oxygen molecules, and GGT, an endogenous antioxidant that facilitates the elimination of xenobiotics and ROS through GSH homeostasis. In patients with rheumatoid arthritis, there is an increase in GGT activity in SF because GGT from lymphocytes or inflammatory cells accumulates in arthritic lesions.[45,46] Another important antioxidant molecule is GST, which facilitates the detoxification of polycyclic aromatic hydrocarbons and benzopyrenes present in tobacco smoke.[47] Evaluation of these antioxidant molecules was important to determine whether smoking significantly interferes with this mechanism and thus promotes the development of OA. In smoking patients under 60 years of age, activation of the antioxidant system, evaluated at the systemic level, was high, as were CAT and GST. These factors were also high in SF. Notably, GGT activity was low in plasma and SF. In those with OA older than 60 years who were smokers, CAT and GGT were high, unlike GST, which was low; however, in SF, the three enzymes were low in the smoking group. These findings suggest that in young adults, despite being smokers, their antioxidant system manages to respond to counteract the OS generated by tobacco consumption; however, this response is not sufficient to avoid the generation of severe OA. In older adults, the system tries to maintain antioxidant molecules at the systemic level, but at the SF level, these molecules do not maintain their functionality, probably due to the duration of tobacco exposure.

Another important enzyme in the structure of the ECM is arginase, which has been considered a biomarker for the progression and severity of some diseases.[48] A recent study showed that arginase promotes disk degeneration associated with the production of OS.[4] The results of this work indicate that plasma arginase activity was high in patients who smoked more than 10 packs per year, but at the joint level, it was low, with a greater degree of pain, suggesting that smoking results in a greater potential for damage, which according to our findings increases GST in smokers as part of the defense mechanism; however, further studies should be conducted to demonstrate the negative effects of smoking at the joint level to establish preventive measures to avoid accelerated cartilage loss.

Although the findings are important, this study has several limitations. First, the small sample size affected the statistical power. Second, we considered only active smokers and former smokers without considering passive smokers; therefore, this study is worth replicating to include this group and increase the sample size to confirm our results. Third, in addition to ROS, tobacco contains toxic metals such as cadmium (Cd), an element that was not included in this study and that has been shown to promote ECM loss in studies using three-dimensional (3D) chondrocyte cultures.[49] Finally, other oxidative molecules, such as nitric oxide, which are involved in the process of articular cartilage loss, as well as inflammatory molecules, such as interleukins, which contribute to the activation of enzymes that degrade ECM and may be related to the severe OA presented by young smokers in this study, should be investigated.

Conclusions

In young smokers, OS is promoted, and the antioxidant system is activated as a defense mechanism; nevertheless, these individuals may develop severe OA, possibly related to higher arginase activity, as arginase is sensitive to cotinine concentrations. In older patients, antioxidant enzyme activity was lower, leading to OS. Our results confirm that

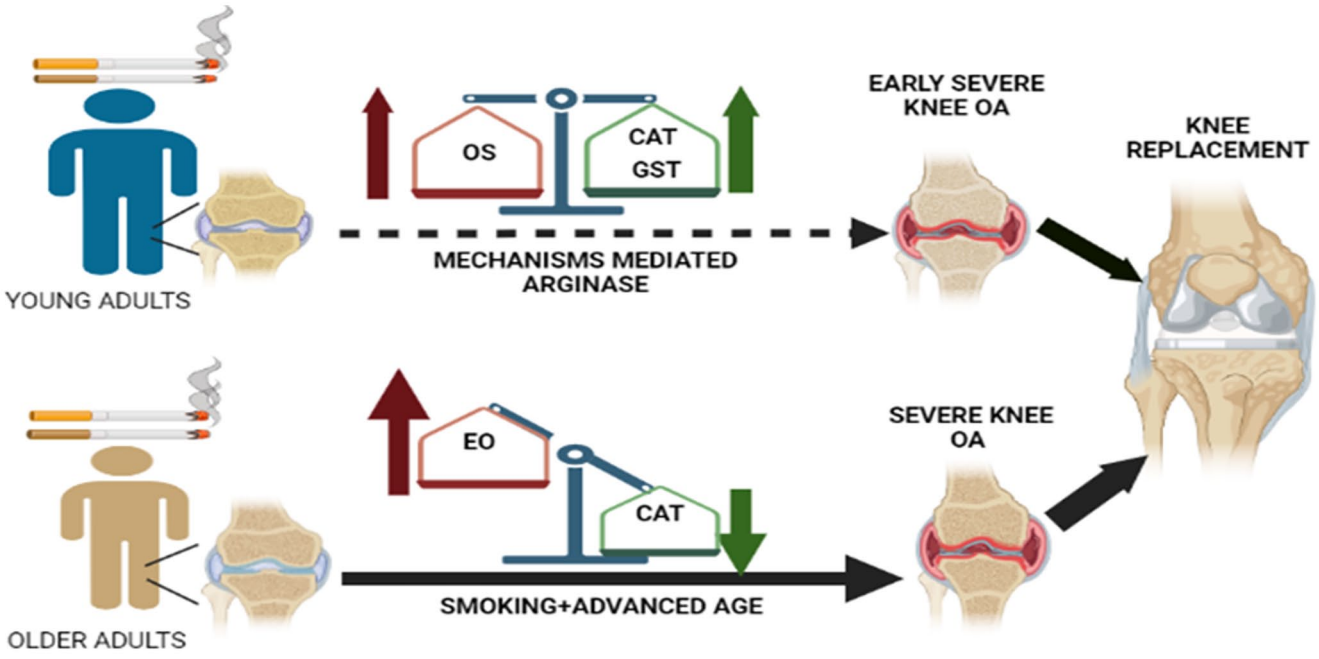

Figure 5. Effect of smoking on the development of knee OA. In young adults who smoke 10 or more packs per year, the early development of severe knee OA is promoted by an increase in OS and arginase activity. In older adults, smoking exacerbates OS by depleting the antioxidant system.

smoking may be an important risk factor for the severity of OA; therefore, knowing its effect on the development of knee OA will allow us to direct prevention strategies to smokers who do not yet require joint replacement (Figure 5).

AUTHORS' CONTRIBUTIONS

JF-T and OGA-A designed the study and the experimental component, analyzed, and interpreted the results to create the manuscript, and gave approval for the final version of the manuscript. YZ-C, AL-M, and RS-S contributed to the analysis of the results at the molecular level. YD-G, AL-R and JN-M supported the analysis of MPO, MGO, APOPs, arginase, GGT, GST, and CAT and reviewed the literature for studies related to quantifying ROS. MPS-V and LMDRL contributed to the structuring and drafting of the manuscript, in particular quantifying ROS. CS-A, VI-S, EJN-C, and AO-M diagnosed OA using radiological assessments and the KL scale. NM-A, IL-J, and BV-S supported the preparation of the samples and reviewed the literature for the discussion of the results. GAM-N provided support for the statistical analysis, contributing vast experience in the statistical analysis of various biological models. KM-F contributed to the study design, experiment oversight, statistical analysis for interpretation of the results, manuscript preparation, and approval of the final revision of the manuscript.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICAL APPROVAL

This study was approved by the Ethics and Research Committee of the Instituto Nacional de Rehabilitación "Luis Guillermo Ibarra Ibarra" (INR-67/19), the Ethics and Research Committee of the Instituto Nacional de Enfermedades Respiratorias "Ismael Cosío Chávez" National Institute of Respiratory Diseases (C33-22), and the Research Ethics Committee of Médica Sur (2021-EXT-551). All the procedures carried out in this study with human participants were conducted in accordance with the ethical standards of the ethics and research committees of the INR-LGII, INER, and Médica Sur and with the Declaration of Helsinki (2013). Informed consent was obtained from all participating individuals included in the study.

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ORCID ID

Karina Martínez-Flores https://orcid.org/0000-0003-0675-0227

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