

Smoking and female sex as key risk factors associated with severe arthralgia in acute and chronic phases of Chikungunya virus infection

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Abstract. Arthralgia is a potentially incapacitating condition and a persistent symptom in chronic or acute episodes of Chikungunya fever caused by infection with the Chikungunya virus (CHIKV). To the best of our knowledge, there are no reports on risk factors associated with the intensity of arthralgias in typical acute episodes of the disease. Although a number of studies have reported on risk factors associated with the development of the chronic stage of the disease, smoking habits have not been analyzed. Smoking is an interesting factor to consider since it is the main environmental risk factor for the development of rheumatoid arthritis (RA), a similar disease to CHIKV in many aspects. In the present study, 140 patients infected with CHIKV were assessed for risk factors associated with severe arthralgia intensity in the acute phase (pain of 9/10 on the visual analog scale of 0-10) and moderate to severe intensity (according to the Routine Assessment of Patient Index Data 3) 3.5 months after infection in patients that experienced the chronic phase of the disease. Women and smokers were 2- to 3-times more likely to experience severe pain in the acute and chronic stages. Likewise, the presence of severe arthralgia during the acute disease phase resulted

in a 4-fold increased risk for entering the chronic phase. Smoking was a more important risk factor in males compared with females. Smoking resulted in a 20-fold increased risk for severe arthralgia during the acute phase in men, as well as a 10-fold increased risk for developing chronic disease with moderate-to-severe pain 3.5 months after the acute stage. The presence of rash, headache, muscular weakness or conjunctivitis in the acute phase, the presence of diabetes and age >40 years were considered significant risk factors due to their influence on illness progression. In conclusion, smoking and female sex were the main risk factors associated with development of severe joint pain in the acute and chronic phases of Chikungunya fever. These risk factors are similar to those associated with the development and severity of RA, possibly because the two diseases share pathophysiological mechanisms, including elevated interleukin-6 levels.

Introduction

Chikungunya fever is a disease caused by the Chikungunya virus (CHIKV), an RNA virus from the genus *Alfavirus* (1). CHIKV is transmitted to humans through the bite of infected *Aedes aegypti* and *Aedes albopictus* mosquitoes (2). These species are prevalent in tropical and subtropical regions, with the potential to affect over one billion people (3). CHIKV infection can present at acute, sub-acute or chronic phase. The acute phase is characterized by a complete disappearance of all symptoms by day 10 from symptom onset, whereas the chronic phase is characterized by symptom persistence of >3 months (4). Symptoms start abruptly in the acute stage of the disease, with high fever (85-100%) and arthralgia (90-98%) that is often disabling (5). Other symptoms include

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headache, muscular weakness, arthritis and dermatological signs (5), as well as digestive disorders, including nausea and vomiting (1-3,5). In the chronic stage, joint inflammation and arthralgia persists. A previous study on 39 patients with chronic CHIKV infection documented a prevalence of chronic inflammatory polyarthralgia in up to 89% of cases (6). In a study conducted in western Mexico, the researchers reported persistent arthralgia in 62.5% of the patients, 3 months after infection. Symptoms prevailed in 42% of these patients at 6 months after infection, whereas they were attenuated in other patients with the passage of time (7).

Studies searching for an association or correlation between molecular, clinical or sociodemographic characteristics and disease severity of CHIKV disease are limited. To the best of our knowledge, only one study has been conducted looking for risk factors associated with illness severity in atypical cases of acute CHIKV disease in adults. The authors identified that hypertension and underlying respiratory or cardiac conditions were independent risk factors for disease severity (8). On the other hand, several studies have identified risk factors associated with the chronic phase of the disease. A recent systematic review analyzed 37 studies and concluded that female sex, older age and high levels of interleukin-6 (IL-6), as well as the severity of the acute phase and certain co-morbidities, were associated with persistent arthralgia (9).

Potential risk factors, including smoking habits, have not been considered in association with CHIKV infection. The study of smoking habits in patients with this disease is of interest, since smoking is one of the most important risk factors known and studied for the development of rheumatoid arthritis (RA). Chronic Chikungunya arthritis symptoms closely mimic RA symptoms and even share certain molecular biomarkers (10), including high levels of IL-6, (11,12), so it can be assumed that the two pathologies may also share key risk factors. The identification of possible similarities between CHIKV arthritis and RA would provide a basis for identifying the pathophysiological and therapeutic molecular mechanisms of the two diseases. The present study searched for risk factors associated with the development of very severe arthralgia in acute and chronic phases of CHIKV infection, with an emphasis on the analysis of smoking habits.

Materials and methods

Ethical approval. This project was approved by the Research Ethics Committee of the Cancerology State Institute, Colima State Health Services (Colima, Mexico) and all participants signed statements of informed consent. Participation in the study was voluntary.

Patient data. The present study included patients with acute CHIKV fever that were admitted to any of the four hospitals of the Health Department of the State of Colima (western region of the Mexican Pacific coast), from July to October 2015. In the acute phase of the disease, patients were evaluated by hospital staff physicians, who filled out standardized clinical records for an epidemiologic study of vector-borne diseases. Personal data, including telephone numbers, and the absence or presence of clinical manifestations, including fever, nausea, vomiting, itching, conjunctivitis, myalgia and arthralgia, were

recorded. Intensity of joint pain was recorded according to the visual analog scale (VAS), from 'no pain' (score of 0) to 'worst imaginable pain' (score of 10) (13). VAS was selected because it is currently the validated scale that best evaluates pain in diseases that manifest arthralgia, including RA (13). Patient's blood samples were sent to the Public Health Laboratory of the Health Department of the State of Colima (Colima, Mexico) for serum analysis and CHIKV diagnostic by using the Trioplex Real-time RT-PCR Assay (Biosystems™ TaqMan™ Arbovirus Triplex Assay; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to manufacturer's instructions for detection and differentiation of RNA from dengue, chikungunya or zika (14).

The study sample included 140 patients ≥ 18 years old (average age, 39.9 ± 14.5 ; 71.4% women) who were positive for CHIKV through RT-qPCR evaluation and provided clinical and personal data during their acute phase, as described above. The patients were also required to be available for a telephone interview 3.5 months after acute infection (chronic phase evaluation). Patients with a self-reported history of systemic rheumatologic disease (RA, multiple sclerosis or systemic lupus erythematosus) were excluded from the analysis. Evaluation of the chronic phase was performed according to the Routine Assessment of Patient Index Data 3 (RAPID3), which is a pooled index of the three patient-reported RA Core Data Set measures of the American College of Rheumatology: Physical function, pain and patient estimate of global status (15). Each of the three individual measures was scored from 0 to 10, for a total of 30 points. Disease severity scores were classified as follows: RAPID3 score, >12 =high; >6 and ≤ 12 =moderate; >3 and ≤ 6 =low; ≤ 3 =remission. Previous findings have correlated RAPID3 scores with the Disease Activity Score 28 and the Clinical Disease Activity Index (15). Additionally, patients were required to provide their most intense arthralgia value (VAS 0-10) within the past 24 h, by telephone, which was set as the arthralgia score for the chronic stage of disease.

Pain evaluation. Pain stratification is a complex topic. Previous studies have attempted to identify pain intensity cutoff points using diverse measuring scales (16-18). However, it has been determined that cutoff points vary, depending on pain type, chronicity, diagnosis, age, co-morbid conditions, cultures and the interference of pain with various activities (including enjoyment of life, daily activity, walking, mood, sleep and work) (19). Thus, VAS scores for pain for classifying patients with severe or very severe pain have varied in different studies (19). In the present study, patients were divided according to their pain in the acute phase, using 9 as the cutoff point (50th percentile of the VAS score). Patients with low arthralgia scores (≤ 8 points) during the acute phase formed the reference group, while patients with higher scores (9 and 10) were considered the study group. The score of 9-10 has been used in previous studies to classify patients with very severe pain or the most severe pain imaginable (20,21). In the chronic phase of the disease, the patients with RAPID3 scores ≤ 6 formed the reference group, whereas patients with scores >6 (moderate/high disease activity) were considered the study group.

Smoking habits. Smoking habits were also evaluated using a definition similar to that established by the CDC National

Health Interview Survey (22). The 100-cigarette threshold has been extensively used to pragmatically identify lifetime smokers (current or former smokers) and differentiate them from experimental or new-onset smokers (23). This classification was also used to evaluate the effect of smoking on the severity of a systemic viral infection causing renal damage (24). In the present study, current smokers were defined as patients that had smoked ≥ 100 cigarettes in their lifetime and that currently smoked daily or occasionally. Ex-smokers were defined as patients that had smoked ≥ 100 cigarettes in their lifetime, but that had not smoked daily or occasionally for ≥ 1 year. Never-smokers were defined as patients that had smoked < 100 cigarettes in their lifetime. According to the questionnaire, the patients were divided into 2 groups to evaluate the effect of current smoking on the severity of acute and chronic phases of CHIKV infection: Current smokers and non-smokers. The group of non-smokers included the ex-smokers and never-smokers. No patients changed their smoking habits during the study.

Statistical analysis. Data are presented as the mean \pm standard deviation. For the inferential statistics, normal data distribution was first determined using the Kolmogorov-Smirnov test and the equality of variances was confirmed using Levene's test. Student's t-test was used to compare the mean values of the normally distributed measurement variables and the homogeneity of variances. Data with non-normal distribution or non-homogeneous variances were evaluated using the Mann-Whitney U test, and groups were compared using the χ^2 test. The association between symptoms in the acute phase of the disease (including headache, rash and muscular weakness), or factors such as sex or smoking habits, and intense arthralgia in the acute (VAS 9/10) or chronic (RAPID3 score, > 6) phase, were estimated by odds ratios (OR) and 95% confidence intervals (CI), using the Crosstabs procedure. The risk factors of sex, smoking and age > 40 years were adjusted through a Cochran-Mantel-Haenszel analysis. For the statistical analysis of disease severity levels obtained from the RAPID3 index, the level of severity was transformed into a numerical value (ordinal scale), where remission=0, mild disease=1, moderate disease=2 and severe disease=3. $P < 0.05$ was considered to indicate a statistically significant difference. All statistical analyses were performed with SPSS version 20 software (IBM Corp., Armonk, NY, USA).

Results

Patient characteristics during acute phase. In total, 53% of patients presented with intense arthralgia during the acute phase of the disease, classified as those with 9-10 points on the VAS. The mean VAS score in the intense arthralgia group was 9.7 ± 0.46 , compared with a mean score of 7.0 ± 1.4 points in the control group. As shown in Table I, females, smokers and patients who were ≥ 40 years old exhibited an increased risk of experiencing high-intensity arthralgia during the acute phase of the disease. This was observed to be a significant increase for female sex (adjusted $P = 0.03$) and smoking (adjusted $P = 0.02$). However, the increased risk for patients who were ≥ 40 years old was not observed to be statistically significant when adjusted for sex and smoking habits.

Patient characteristics during chronic phase. A notable finding was that 3.5 months after CHIKV infection, 68% of the patients had entered either a moderate or severe chronic stage of the disease, according to the RAPID3 multidimensional health assessment questionnaire. For patients with chronic disease, the mean VAS score for arthralgia was 5.7 ± 2.8 , compared with 0.9 ± 1.4 for the control group (patients with RAPID3 values ≤ 6) ($P < 0.001$). A total of 78% of patients with moderate/severe chronic disease and 24% of patients with mild chronic disease, or in remission, used non-steroidal anti-inflammatory drugs (NSAIDs) for pain relief. Paracetamol was the most widely prescribed pain relief medication among all participants (70%). Anti-rheumatic drugs were not registered for any of the patients of the present study.

Risk factors for entering chronic phase. As indicated in Table II, female sex, smoking and the presence of severe arthralgia in the acute phase of the Chikungunya fever were risk factors associated with developing a severe chronic stage. Statistical adjustment of the data (for sex and smoking) confirmed these risk factors (female, adjusted $P = 0.005$; smoking, adjusted $P = 0.03$; severe arthralgia in acute phase, $P = 0.002$). The adjusted analysis also demonstrated that the presence of exanthema during the acute phase of disease increased the risk of entering chronic stage (adjusted $P = 0.02$), whereas conjunctivitis reduced the risk of entering chronic stage (adjusted $P = 0.04$).

Risk factors for severe pain. Table III indicates the mean values for arthralgia intensities (VAS scores) in patients during the acute and chronic stages of Chikungunya fever, as well as the RAPID3 level of chronic disease severity, according to the presence or absence of certain clinical characteristics in the acute phase of the disease. It was evident that patients that smoked, as well as patients of the female sex, experienced significantly higher pain intensity during the acute phase of disease ($P = 0.003$). Likewise, patients with severe arthralgia in the acute phase experienced significantly higher arthralgia 3.5 months after infection ($P = 0.001$), as well as more severe chronic disease ($P < 0.001$). Patients ≥ 40 years of age did not exhibit a significant increase in pain during the acute phase of the disease or in severity of chronic disease, but did exhibit a significant increase in pain during the chronic stage ($P = 0.004$). Patients with exanthema in the acute phase did not experience a significant increase in pain intensity during the chronic stage, despite what was observed in Table II. The presence of conjunctivitis was associated with a significant decrease in the severity of chronic disease ($P = 0.04$). However, patients with conjunctivitis did not exhibit a significant reduction in pain intensity ($P = 0.58$).

Patients that experienced headache and muscular weakness during the acute phase also presented, with significantly more intense, arthralgia in the acute and chronic stages. Furthermore, the severity of chronic disease was significantly higher in patients that experienced headache ($P = 0.02$; Table III). In patients with diabetes, arthralgia was significantly more intense during the chronic stage.

Hormonal status of female patients did not significantly modify the risk of experiencing intense pain during the acute phase (OR, 1.5; 95% CI, 0.6-3.7; $P = 0.22$) or severe chronic

Table I. Association between clinical characteristics of patients and high-intensity arthralgia during the acute phase of Chikungunya infection.

Characteristic	Controls, % (n=66)	Cases, % (n=74)	Crude OR (95% CI)	P-value	Adjusted OR ^a (95% CI)	Adjusted P-value
Smoking	13.6	35.1	3.43 (1.46-8.02)	0.004 ^b	3.03 (1.16-7.86)	0.020 ^b
Female sex	62.1	79.7	2.30 (1.12-5.09)	0.020 ^b	2.42 (1.04-5.58)	0.030 ^b
Age ≥40 years	42.4	59.4	1.99 (1.01-3.90)	0.040 ^b	1.44 (0.67-3.10)	0.340
Diabetes	10.6	12.1	1.16 (0.40-3.33)	0.770	0.52 (0.11-2.34)	0.390
Obesity	30.3	39.1	1.48 (0.73-2.99)	0.270	1.32 (0.58-3.00)	0.580
Hypertension	19.6	12.1	0.56 (0.22-1.42)	0.220	0.36 (0.12-1.05)	0.060
Exanthema	45.4	45.9	1.00 (0.52-1.98)	0.950	1.55 (0.68-3.52)	0.290
Conjunctivitis	9.0	12.1	1.38 (0.46-4.12)	0.580	1.55 (0.41-5.75)	0.510
Pruritus	24.2	25.6	1.07 (0.50-2.32)	0.840	1.35 (0.52-3.49)	0.530
Muscular weakness	1.5	9.4	6.79 (0.81-56.74)	0.070	3.70 (0.39-35.17)	0.250
Headache	93.3	98.6	4.70 (0.51-43.24)	0.170	2.50 (0.26-25.34)	0.410
Retroocular pain	63.6	60.8	0.88 (0.44-1.75)	0.730	0.74 (0.32-1.66)	0.460
Photophobia	19.6	17.5	0.86 (0.37-2.03)	0.740	1.42 (0.49-4.06)	0.510
Nasal congestion	1.5	6.7	4.70 (0.53-41.40)	0.160	ND	
Arthritis	48.4	40.5	0.72 (0.37-1.41)	0.340	0.67 (0.30-1.50)	0.330
Vomiting	13.6	9.4	0.66 (0.23-1.88)	0.440	0.60 (0.19-1.93)	0.400
Nausea	28.7	37.8	1.50 (0.74-3.06)	0.250	1.71 (0.73-3.9)	0.210
Diarrhea	10.6	4.0	0.35 (0.08-1.43)	0.140	0.57 (0.11-2.99)	0.510
Abdominal pain	16.6	10.8	0.60 (0.22-1.61)	0.310	0.62 (0.18-2.16)	0.450
Dysgeusia	10.6	13.5	1.31 (0.47-3.68)	0.320	2.23 (0.60-8.25)	0.220

Symptoms are those suffered by patients in the acute phase. P-values were calculated using the χ^2 test. ^aAdjusted for the variables of smoking (yes/no), sex (male/female) and age ≥40 years (yes/no) through the Cochran-Mantel-Haenszel method. In a case, data were considered ND due to very small or lack of data for different strata of adjusted analysis. ^bP<0.05. OR, odds ratio; CI, confidence intervals; ND, not determined.

disease (OR, 1.6; 95% CI, 0.5-4.7; P=0.25), as observed from patients with menopause (data not shown).

Effects of smoking habits. Smoking habits modified the course of the disease differently in men and women. As indicated in Table IV, smoking increased pain intensity in the acute and chronic stages of the disease in males and females. However, this increase was only observed to be significant in males (P<0.001 at acute stage; P=0.003 at chronic stage). Arthralgia scores during the acute or chronic stage of the infection were similar among male and female smokers. However, in male and female non-smokers, males exhibited a significantly lower risk of experiencing arthralgia at the acute and chronic stages (both P<0.001). Mean arthralgia values in the chronic phase for male non-smokers were 62% less intense compared with male smokers. On the other hand, pain intensity was reduced by only 15% in female non-smokers compared with female smokers. These data indicate that smoking, in relation to arthralgia intensity in CHIKV infection, affected males to a greater extent than females, and that arthralgia pain intensity was generally stronger in male smokers. Male smokers exhibited a 20-fold increased risk for experiencing very intense pain during the acute phase (OR, 20.1 95% CI, 2.1-189.8; P=0.003), and a 10-fold increased risk for entering a moderate/severe chronic phase after 3.5 months, compared with non-smokers (OR, 10.5; 95% CI, 1.1-96.5; P=0.02).

In agreement with these results, severity of arthritis due to CHIKV infection was significantly increased by smoking habits, according to RAPID 3 scores, which was only indicated in male smokers (P=0.04, Table IV).

Discussion

Smoking and female sex are the key risk factors for experiencing severe arthralgia during the acute and chronic phases of CHIKV infection. There are no previous reports associating smoking habits with the severity of symptoms during CHIKV infection, but it is logical to assume that smoking can influence the severity of a viral infection that affects the lungs, since they could already be compromised by the chemical components of cigarette smoke. This premise has previously been demonstrated in association with influenza infection (25). Smoking has also been demonstrated to influence the severity of viral infections in other organs. For example, smoking has recently been reported to be associated with aggravated kidney damage in Puumala hantavirus-induced hemorrhagic fever with renal syndrome (24).

Smoking can also influence viral pathogenesis in numerous ways. For instance, viral replication of the respiratory syncytial virus has been reported to increase in cell cultures previously exposed to cigarette smoke extract (26). Additionally, cigarette smoke reduces the expression of interferon-inducible genes,

Table II. Association between clinical characteristics of patients and moderate/severe disease in the chronic phase of Chikungunya infection.

Characteristic	Controls, % (n=45)	Cases, % (n=95)	Crude OR (95% CI)	P-value	Adjusted OR ^a (95% CI)	Adjusted P-value
Smoking	13.3	30.5	2.85 (1.08-7.48)	0.030 ^c	2.93 (1.05-8.14)	0.030 ^c
Female sex	55.5	78.9	3.0 (1.39-6.46)	0.005 ^c	3.13 (1.40-6.98)	0.005 ^c
≥ 40 years	44.4	54.7	1.51 (0.74-3.08)	0.250	1.51 (0.70-3.23)	0.280
Severe arthralgia ^b	28.8	64.2	4.41 (2.04-9.52)	<0.001 ^c	3.65 (1.57-8.44)	0.002 ^c
Diabetes	8.8	12.6	1.48 (0.45-4.88)	0.510	1.43 (0.36-5.63)	0.600
Obesity	26.6	38.9	1.75 (0.80-3.82)	0.150	1.57 (0.67-3.67)	0.290
Hypertension	11.1	17.8	1.74 (0.59-5.07)	0.300	1.36 (0.46-4.02)	0.570
Exanthema	33.3	51.5	2.01 (0.96-4.22)	0.060	3.07 (1.17-8.03)	0.020 ^c
Conjunctivitis	17.7	7.3	0.36 (0.12-1.08)	0.070	0.26 (0.07-0.94)	0.040 ^c
Pruritus	17.7	28.4	1.83 (0.75-4.44)	0.170	2.88 (0.89-9.29)	0.070
Muscular weakness	2.2	7.3	3.50 (0.41-29.34)	0.240	ND	
Headache	93.3	97.8	3.32 (0.53-20.62)	0.190	7.32 (0.54-98.11)	0.130
Retroocular pain	66.6	60.0	0.75 (0.35-1.57)	0.440	0.66 (0.27-1.59)	0.350
Photophobia	24.4	15.7	0.57 (0.24-1.39)	0.220	0.91 (0.32-2.56)	0.860
Nasal congestion	2.2	5.2	2.44(0.27-21.56)	0.420	ND	
Arthritis	44.4	44.2	0.99 (0.48-2.02)	0.970	0.78 (0.33-1.86)	0.580
Vomiting	13.3	10.5	0.76 (0.25-2.25)	0.620	0.41 (0.11-1.48)	0.170
Nausea	28.8	36.8	1.43 (0.66-3.09)	0.350	1.76 (0.69-4.49)	0.230
Diarrhea	4.4	7.3	1.71 (0.34-8.58)	0.510	2.30 (0.43-12.14)	0.320
Abdominal pain	8.8	15.7	1.92 (0.59-6.16)	0.270	1.72 (0.42-6.98)	0.440
Dysgeusia	15.5	10.52	0.63 (0.22-1.80)	0.390	0.78 (0.22-2.72)	0.700

Symptoms are those suffered by patients in the acute phase. P-values were calculated using the χ^2 test. ^aAdjusted for the variables of smoking (yes/no) and sex (male/female) through the Cochran-Mantel-Haenszel method. ^bSevere arthralgia (visual analog scale 9-10) during acute phase. ^cP<0.05. OR, odds ratio; CI, confidence intervals. In some cases, data were considered ND due to very small or lack of data for different strata of adjusted analysis. ND, not determined.

in turn, limiting the antiviral responses, in a neonatal mouse model (27). This evidence is consistent with the increased severity of CHIKV infection observed in patients that smoked in the present study, particularly regarding arthralgia intensity in the acute phase of illness.

IL-6 has been demonstrated to be involved in the pathophysiology of CHIKV infection. Increased IL-6 levels are associated with increased severity and persistence of joint pain (4). Notably, cigarette smoke extract in non-cytotoxic doses is capable of increasing the levels of IL-6 in cell cultures (28) and in animal models (29). Additionally, smoking correlates with higher serum levels of IL-6 in apparently healthy individuals, the elderly and HIV-infected patients (30-32). An increase in pre-existing IL-6 levels in smokers could be an important cause of symptom severity in smokers that acquire CHIKV infection. Smoking has been observed to affect the immune response at the cellular and humoral level, hence it could induce proinflammatory and immunosuppressive effects (33). Thus, additional immunological alterations may be producing general and severe symptoms in patients that are smokers.

It is widely established that chronic sequelae of CHIKV infection mimic RA (34). For example, high levels of IL-6 are associated with inflammation and joint damage in RA patients (35). Thus, the two pathologies share a clinical profile

and high levels of IL-6. In addition, smoking is the main environmental risk factor for developing RA and is also associated with promoting early stages and higher severity of the disease. For these reasons, in the present study, it was investigated whether smoking is a shared risk factor with Chikungunya infection arthralgia. Notably, serum levels of ILs (including IL-6) in ex-smokers have been reported to not be significantly reduced, even 4 months after quitting smoking (36). Therefore, the severity of arthralgia caused by CHIKV infection in smokers may not disappear until many months after quitting. This requires further exploration in future investigations.

To the best of our knowledge, no previous studies have established an association between arthralgia intensity during the acute phase of typical CHIKV infection and the subsequent symptomatology in patients that progress into chronic stage. Nevertheless, previous reports associate many risk factors with arthralgia persistence. A recent systematic review analyzed 37 studies and concluded that female sex, older age and high levels of IL-6, as well as the severity of the acute phase and some co-morbidities, were associated with persistent arthralgia (37). These clinical results are consistent with those reported in the present study: Female sex as a risk factor for experiencing high-intensity arthralgia during the acute and chronic stages of the disease; a strong tendency for both

Table III. Arthralgia intensity in the acute and chronic phases of Chikungunya infection, according to the absence or presence of clinical characteristics.

Clinical characteristic	VAS score of arthralgia during acute phase			VAS score of arthralgia during chronic phase			RAPID3 score of chronic disease severity		
	Presence	Absence	P-value	Presence	Absence	P-value	Presence	Absence	P-value
Smoking	9.1±1.3	8.2±1.7	0.003 ^b	5.4±3.0	3.9±3.3	0.020 ^b	2.4±0.9	1.8±1.3	0.040 ^b
Female	8.7±1.4	7.6±2.0	0.003 ^b	4.8±3.1	2.9±3.3	0.003 ^b	2.2±1.2	1.4±1.3	<0.001 ^b
Age ≥40 years	8.5±1.7	8.1±1.6	0.070	5.2±3.0	3.5±3.3	0.004 ^b	2.2±1.2	1.8±1.3	0.070
Severe arthralgia ^a	9.7±0.4	7.0±1.4	<0.001 ^b	5.1±3.1	3.2±3.3	0.001 ^b	2.4±1.0	1.5±1.3	<0.001 ^b
Diabetes	8.4±2.0	8.4±1.6	0.560	6.2±3.3	4.0±3.2	0.020 ^b	2.5±0.9	1.9±1.3	0.170
Obesity	8.5±1.6	8.3±1.7	0.440	4.6±3.0	4.1±3.4	0.400	2.2±1.2	1.8±1.3	0.140
Hypertension	8.3±1.7	8.4±1.6	0.770	5.2±3.3	4.1±3.3	0.150	2.3±1.0	1.9±1.3	0.170
Exanthema	8.4±1.4	8.4±1.9	0.540	4.7±3.4	4.4±3.2	0.680	2.2±1.1	2.0±1.3	0.670
Conjunctivitis	8.1±2.5	8.4±1.6	0.750	4.0±3.4	4.6±3.2	0.580	1.5±1.3	2.2±1.2	0.040
Pruritus	8.4±1.6	8.4±1.7	0.910	5.3±3.1	4.3±3.3	0.150	2.4±1.1	2.0±1.2	0.140
Muscular weakness	9.5±0.7	8.3±1.7	0.040 ^b	8.4±1.5	4.2±3.2	0.001 ^b	2.7±0.6	2.0±1.2	0.080
Headache	8.5±1.5	6.2±3.0	0.050	4.4±3.2	1.8±2.4	0.070	2.0±1.2	0.6±1.3	0.020 ^b
Photophobia	8.2±2.1	8.4±1.6	0.970	4.1±3.1	4.6±3.3	0.530	1.9±0.3	1.2±1.1	0.770
Retroocular pain	8.5±1.5	8.2±2.0	0.610	4.7±3.2	4.2±3.3	0.380	2.0±1.2	2.1±1.3	0.680
Nasal congestion	9.5±0.5	8.3±1.7	0.190	7.2±1.2	4.4±3.3	0.090	2.5±0.5	2.0±1.2	0.910
Arthritis	8.4±1.7	8.3±1.6	0.640	4.3±3.2	4.7±3.3	0.560	2.0±1.2	2.1±1.2	0.720
Vomiting	8.2±1.8	8.4±1.7	0.840	5.0±3.2	4.4±3.2	0.520	1.9±1.1	2.1±1.2	0.400
Nausea	8.6±1.6	8.3±1.7	0.350	4.6±3.2	4.4±3.3	0.800	2.2±1.1	2.0±1.2	0.470
Diarrhea	7.3±2.5	8.5±1.6	0.180	3.8±3.7	4.6±3.2	0.560	2.4±1.1	2.0±1.2	0.340
Abdominal pain	8.2±1.6	8.4±1.7	0.540	5.2±3.2	4.4±3.2	0.350	2.5±1.0	2.0±1.2	0.120
Dysgeusia	8.4±2.2	8.4±1.6	0.520	4.3±3.4	4.5±3.2	0.710	1.8±1.4	2.1±1.2	0.580

Data are presented as the mean ± standard deviation. Arthralgia was quantified using VAS scores, from 'no pain' (score of 0) to 'worst pain imaginable' (score of 10). Severity chronic disease was quantified using the RAPID3 index by transforming the severity level into a numerical value (ordinal scale), where remission=0, mild disease=1, moderate disease=2 and severe disease=3. ^aSevere arthralgia (VAS 9-10) during acute phase. P-values were calculated by performing the Mann-Whitney U test. ^bP<0.05. VAS, visual analog scale; RAPID3, Routine Assessment of Patient Index Data 3.

Table IV. Arthralgia intensity in the acute and chronic phases of Chikungunya infection, according to the sex and smoking habits of patients.

Sex	VAS score of arthralgia during acute phase			VAS score of arthralgia during chronic phase			RAPID3 score of chronic disease severity		
	Smoker	Non-smoker	P-value	Smoker	Non-smoker	P-value	Smoker	Non-smoker	P-value
Male n=40	9.2±0.9	7.2±2.0	<0.001 ^a	5.6±3.4	2.1±2.8	0.003 ^a	2.1±0.9	1.2±1.3	0.040 ^a
Female n=100	9.0±1.3	8.6±1.3	0.080	5.4±2.7	4.6±3.2	0.140	2.4±0.9	2.1±1.2	0.110
P-value	0.350	<0.001 ^a		0.440	<0.001 ^a		0.200	<0.001 ^a	

Data are presented as the mean ± standard deviation. Arthralgia was quantified using VAS scores, from 'no pain' (score of 0) to 'worst pain imaginable' (score of 10). Severity of chronic disease was quantified using the RAPID3 index by transforming the severity level into a numerical value (ordinal scale), where remission=0, mild disease=1, moderate disease=2 and severe disease=3. P-values were calculated by performing the Mann-Whitney U test. ^aP<0.05. VAS, visual analog scale; RAPID3, Routine Assessment of Patient Index Data 3.

sexes to go into a moderate or severe chronic stage if there was intense arthralgia during the acute phase; and an increased

risk for high-intensity arthralgia during the chronic stage in patients ≥40 years of age.

According to results observed in the present study, for Chikungunya fever in general, female sex and smoking were the most important risk factors that induced a disease with severe symptomatology. This trend is also observed in rheumatoid arthritis, with females exhibiting worse progression compared with males (38). The inflammatory response has been demonstrated to differ between the sexes. A clinical study recently revealed a greater proinflammatory response in females, with significantly increased concentrations of tumor necrosis factor α and IL-6 in the plasma compared with males, when exposed to a bacterial endotoxin. Consequently, a greater proinflammatory response, particularly elevated IL-6 levels, could cause women to experience more severe symptomatology in CHIKV infection, as well as in RA (39).

Smoking also affects men and women differently. The present study demonstrated that male patients that smoked experienced more severe arthralgia in the acute and chronic phases of Chikungunya infection compared with male non-smokers, while female smokers were not significantly different from female non-smokers. Furthermore, in a meta-analysis of 16 studies on patients that smoked, it was identified that male smokers exhibited a summary OR of 1.87 (95% CI, 1.49-2.34) for developing RA and a summary OR of 3.91 (95% CI, 2.78-5.50) for positive rheumatoid factor. Furthermore, the same meta-analysis demonstrated that female smokers had respective OR values of 1.31 (95% CI, 1.12-1.54) and 1.29 (95% CI, 0.94-1.77) (40). Thus, smoking was a greater risk factor for developing RA in males compared with females, illustrating another similarity between Chikungunya fever and RA.

Patient age was also a significant risk factor in the present study, given that patients ≥ 40 years of age presented with the highest arthralgia scores. In RA, an adjusted multivariate analysis that included 197 patients demonstrated that late-onset patients presented with greater disease activity (41). This could be associated with an increased concentration of proinflammatory ILs, including IL-6, in the blood of older people (42), which could induce more severe arthralgia in the two diseases. The association between patient age and severity of symptoms is another shared characteristic of Chikungunya fever and RA.

The present study also identified that diabetes and the presence of rash, muscular weakness, headache and conjunctivitis during the acute phase of the disease are associated with the disease course. Likewise, two previous studies have associated diabetes or rash with persistent arthralgia after CHIKV infection (43,44). By contrast, to the best of our knowledge, the occurrence of conjunctivitis during the acute phase as a risk factor for the severity of arthralgia, or headache and muscular weakness as intensity-increasing factors, have not been previously reported.

It is important to emphasize that two different types of statistical analyses were used to interpret arthralgia intensity in the present study: Association of risk factors and comparison of means. The two analyses identified smoking and female sex as relevant risk factors. They were consistent in identifying that a severe acute phase of the disease leads to a severe chronic stage. These variables were the most important in determining the clinical course of the disease. The other symptoms of headache, conjunctivitis, muscular weakness and rash were considered less relevant risk factors, since they were significant in only one statistical analysis. This also implies

that the differences between studies on CHIKV infection could be due not only to real differences in disease progression, but also to the type of statistical strategy applied.

A key limitation of the present study was that a single arthralgia intensity score (described by the patient as the most intense pain at the time of the evaluation) was considered for the analysis of arthralgia severity in the chronic phase, regardless of whether such pain was being experienced in >1 joint. Furthermore, a multidimensional analysis (RAPID3 scale) that has not appeared in studies in the literature was applied to evaluate the general state of patients during the chronic stage of disease.

It was clear from the results of the current study that smoking, female sex, or having experienced severe arthralgia in the acute phase of CHIKV infection are the primary factors that promote severe sequelae, particularly high-intensity arthralgia. Therefore, it is important to conduct studies that evaluate whether the application of effective antiviral or anti-arthralgia treatments (45) during the acute phase of the disease will reduce the sequelae or severity of infection. In the present study, treatment in the chronic phase was symptomatic and based on NSAIDs. However, the similarity between CHIKV and RA arthralgias has guided some physicians to use methotrexate or anti-tumor necrosis factor agents (standard treatments for RA) in patients with chronic Chikungunya syndrome, with good response and tolerance (46). Despite the success observed in controlled studies, those treatments are not yet recommended in the international clinical management guidelines for patients with CHIKV (47). In the present study, similarities in risk factors for CHIKV arthralgia and RA development were identified or confirmed. Such risk factors are generally supported by common pathophysiological mechanisms, particularly elevated IL-6 levels, so these findings provide a basis for identifying common pathophysiological and therapeutic mechanisms in the two diseases.

The results of the present study could aid physicians in regions that are endemic for CHIKV. The findings prompt more detailed follow-up in female patients and smokers that are infected with the disease, given that they present with a higher risk of intense and chronic illness. At the public health level, these results contribute to a greater understanding of increased symptomatology in infectious diseases in individuals exposed to smoke. The findings provide additional support for strategies that focus on reducing smoke exposure.

In conclusion, smoking and female sex are the key risk factors for presenting with severe joint pain in acute and chronic phases of CHIKV infection. Risk factors for increased severity and chronicity of arthralgia in CHIKV appear to be similar to those associated with the development and severity of RA, which may be due to their shared pathophysiological mechanisms. To the best of our knowledge, this is the first study to associate smoking with the clinical course of CHIKV infection, and therefore more studies are required in this field.

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