Burden of depressive symptoms in South African public healthcare patients with established rheumatoid arthritis: a case-control study

A. Solomon[1], B.F. Christian[1], A.J. Woodiwiss[2], G.R. Norton[2], P.H. Dessein[1,2]

[1]Department of Rheumatology, University of the Witwatersrand and Charlotte Maxeke Johannesburg Academic Hospital, Johannesburg, South Africa; [2]Cardiovascular Pathophysiology and Genomics Research Unit, School of Physiology; Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

Abstract

Objective

The burden of depressive symptoms and how demographic and disease characteristics relate to depressive symptoms in patients with rheumatoid arthritis (RA) that belong to developing populations, are currently unknown and were therefore assessed in a case-control study in public healthcare patients in South Africa, a lower-middle income country. Public healthcare attendance is a surrogate of belonging to the developing population in South Africa.

Methods

Demographic and RA features were recorded in 441 public and 202 private healthcare patients. The outcome characteristic was the Arthritis Impact Measure Scales (AIMS) depression score. Relationships of patient characteristics and public healthcare attendance with depressive symptoms were determined in multivariable regression models.

Results

The mean ± SD AIMS depression score was 3.6±2.1 and 2.3±1.7 in public and private healthcare patients, respectively ($p<0.0001$ before and after adjustment for covariates). Physical disability was associated with depressive symptoms in both healthcare sectors. Other characteristics that were related to depressive symptoms comprised younger age, male sex and pain in public healthcare patients and fatigue and non-use of disease modifying agents in private healthcare patients. In all patients, public healthcare attendance (standardised ß [95% CI]=0.22 [0.12, 0.32], $p<0.0001$) and physical disability (standardised β [95% CI]=0.25 [0.16, 0.34], $p<0.0001$) were most strongly associated with depressive symptoms.

Conclusion

The burden of depressive symptoms is markedly enhanced in our developing population with RA, independent of age, sex, ethnic origin and disease characteristics. In this setting, the role of social factors should be assessed and, despite restricted resources, depressive symptoms should be routinely addressed.

Key words

rheumatoid arthritis, depressive symptoms, developing populations

Ahmed Solomo, MBBCH, FCP(SA)
Berenice F. Christian, MBBCH, FCP(SA)
Angela J. Woodiwiss, PhD
Gavin R. Norton, MBBCH, PhD
Patrick H. Dessein, MD, FCP(SA), FRCP(UK), PhD

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Please address correspondence and reprint requests to:
Dr Patrick H. Dessein,
P. O. Box 1012,
Melville 2109,
Johannesburg, South Africa
E-mail: dessein@telkomsa.net

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Introduction

Depression is reportedly two to three times more prevalent in patients with rheumatoid arthritis (RA) than in the general population (1-6). Depression, along with other parameters, forms an integral part of the disablement process (7, 8) and impacts substantially on numerous disease outcomes in RA (1, 2, 4, 9, 12, 13). The most studied risk factors for depression in RA include the demographic features of age and sex, disease activity and severity as well as pain and fatigue (1, 2, 4-6). Additionally, social factors can affect depressive symptoms in RA (2, 6, 14).

Depression is currently the fourth leading cause of disease burden (15) and causes the largest amount of non-fatal disease burden (16) worldwide. This is probably an underestimate because of an inadequate appreciation of the connectedness between mental illness and other healthcare conditions (15, 17). Although more than 85% of the world's population lives in 153 low and middle income countries (18), only 6% of the research on mental health was published in indexed journals from these countries by 2007 (19). In line with this observation, the burden and potential determinants of depressive symptoms in patients with RA that live in developing countries are currently unknown. Importantly in the present context, knowledge on mental health that is derived from developed countries may not be generalisable (16, 17, 20-22).

South Africa is a lower-middle income country (23). However, it is characterised by vast socioeconomic inequalities (24, 25), now has a Gini coefficient index of 0.679 (the closer to 1, the greater the inequality) and, indeed, has become the most unequal society in the world (26). Consequently, a minority of South Africans follows a westernised life style and mostly lives in modern cities, owns a private sector medical scheme and attends the private healthcare sector (27, 28). By contrast, the vast majority of South Africans follow more traditional lifestyles, live outside of these cities, do generally not have the resources required to own a private sector medical scheme and therefore seek help in the public healthcare sector in which the annual expenditure per head is 6.8-fold less than in the private healthcare sector (27, 28). Taken together, reported evidence indicates that public and private healthcare attendance constitute surrogates of belonging to the developing and developed population in South Africa, respectively (24, 25, 27-29).

In 2005, we reported a markedly higher Health Assessment Questionnaire (HAQ) disability index in South African public compared to private healthcare patients with RA (29). This called for more regular and comprehensive disease monitoring in order to obtain tighter RA control (29). As part of our undertaking and after obtaining approval by the Ethics Committee on Human Subjects (Medical) of the University of the Witwatersrand, a range of patient characteristics that included the Arthritis Impact Measurement Scales (AIMS) depression score (12) have been prospectively and systemically recorded at each clinic visit in patients with RA that were seen by us (AS, BFC and PHD). All patients are invited to give written informed consent to employ the recorded data for future research with less than 1% of them refusing to participate. The present investigation represents a cross-sectional analysis of the data obtained in unselected patients with RA in order to address whether the burden of depressive symptoms and the relationships of demographic and RA characteristics with depressive symptoms are different in South Africans with RA that belong to developing compared to developed populations.

Patients and methods

Study population

Public healthcare patients were seen by AS and BFC at the Charlotte Maxeke Johannesburg Academic Hospital and private healthcare patients by PHD at the Milpark Hospital in Johannesburg. Each patient met the American College of Rheumatology criteria for rheumatoid arthritis (30) and had been prescribed disease modifying agents for rheumatic disease (DMARDs), which indicates that they had established disease.

Assessments

We recorded the demographic charac-

teristics of age, sex and ethnic origin, RA duration, rheumatoid factor status, disease activity, physical disability, disease severity, pain, fatigue and RA treatment including the use of disease-modifying anti-rheumatic drugs (DMARDs), non-steroidal anti-inflammatory agents (NSAIDs), prednisone and biologic therapies. Disease activity was estimated by the Disease Activity Score in 28 joints (DAS28) as calculated from tender and swollen joint counts, a patient global disease activity rating scale and the erythrocyte sedimentation rate (ESR) (31). The Stanford Health Assessment Questionnaire disability index (HAQ score) was used to assess physical disability and impaired function (31). Additionally, the number of deformed joints was recorded as a disease severity marker (32). Pain and fatigue were measured by employing visual analogue scales (VAS). Patients selected points on 10 cm lines where 1 and 10 denoted no and extreme pain or fatigue, respectively.

We used the AIMS depression score as the outcome measure in this study (12, 33). It comprises 5 items and has a range from 5 to 25 that is then normalized to a 0 to 10 range. The AIMS depression score has previously documented reliability and validity (12, 33). Only a clinical interview can establish whether a patient has depression or not. However, the finding of an AIMS depression score of ≥4 indicates that a patient could be assessed as being depressed at interview (12).

Statistical analyses

Disease duration was logarithmically transformed prior to statistical analysis in view of its non-normal distribution. Demographic and RA characteristics (Table I) and AIMS depression scores were compared between public and private healthcare patients using Student *t*-tests or chi-square tests as appropriate. Subsequently, univariate associations of demographic and RA characteristics with depression scores were determined by Pearson correlation coefficients (Table II) or chi-square tests (Table III) separately in public and private healthcare patients. Demographic and RA characteristics that were associated with depressive symptoms or/and differed by healthcare setting in univariate analysis were then entered in multivariable linear regression models in order to determine which variables were independently associated with the AIMS depression scores in public and private healthcare patients, respectively; ethnic origin was forced into the models (Table IV). The independent association of public healthcare attendance and demographic and RA characteristics with the AIMS depression score in all public and private healthcare patients was assessed in a separate multivariable linear regression model (Table V). Finally, the prevalence of an AIMS depression score of ≥4 was compared in a univariate and a potential confounding variable adjusted logistic regression model between public and private healthcare patients (Fig. 1). Statistical computations were made using the GB Stat TM program (Dynamic Micro Systems, Inc., Silverspring, Maryland, USA). Significance was set at 5%.

Table I. Baseline characteristics in all, public and private healthcare patients with RA*.

Characteristic	All (n=643)	Public (n=441)	Private (n=202)	*p*-value
Age, mean ± SD years	55.8 ± 11.7	55.5 ± 11.5	56.2 ± 12.1	0.5
Women	545 (84.8)	383 (86.9)	162 (80.2)	0.03
Ethnic origin				
Black	304 (47.2)	295 (66.9)	9 (4.5)	<0.0001
White	228 (35.5)	60 (13.6)	168 (83.2)	<0.0001
Asian	65 (10.1)	49 (11.1)	16 (7.8)	0.3
Mixed ancestry	46 (7.2)	37 (8.4)	9 (4.5)	0.08
RA duration, mean ± SD years†	9.2 ± 2.4	9.1 ± 2.4)	9.4 ± 2.4	0.7
RF positive	488 (76.0)	330 (75.0)	158 (78.2)	0.05
DAS28, mean ± SD	2.9 ± 1.5	3.2 ± 1.5	2.4 ± 1.4	<0.0001
HAQ score, mean ± SD	0.72 ± 0.66	0.83 ± 0.66	0.47 ± 0.58	<0.0001
N deformed joints, mean ± SD	5.2 ± 8.0	10.0 ± 9.1	5.2 ± 8.0	<0.0001
VAS pain (0-10), mean ± SD	3.6 ± 3.1	4.3 ± 3.1	2.6 ± 2.6	<0.0001
VAS fatigue (0-10), mean ± SD	3.2 ± 2.7	3.8 ± 2.9	3.2 ± 2.7	0.03
RA treatment				
DMARDs	629 (97.8)	439 (99.5)	190 (94.1)	0.0006
NSAIDs	122 (19.0)	78 (17.7)	44 (21.8)	0.2
Prednisone	34 (5.3)	20 (4.3)	14 (6.9)	0.2
Biologic therapies	6	0 (0)	6 (3)	0.0009

*Values are the number (percentage) unless otherwise indicated. RA: rheumatoid arthritis; RF: rheumatoid factor; DAS28: Disease Activity Score in 28 joints; HAQ: Health Assessment Questionnaire; N: number; VAS: visual analogue scale; DMARDs: disease modifying agents for rheumatic disease; NSAIDs: non-steroidal anti-inflammatory drugs.
†Geometric means and SD are given since this characteristic had a non-normal distribution.

Table II. Pearson correlations among depression scores and demographic and RA characteristics in public and private healthcare patients.

	Depression score			
	Public		Private	
Characteristic	r	*p*-value	r	*p*-value
Age, years	-0.082	0.09	0.013	0.9
Log RA duration, years	0.060	0.2	-0.058	0.4
DAS28	0.157	0.001	0.288	<0.0001
HAQ score	0.299	<0.0001	0.366	<0.0001
N deformed joints	0.100	0.02	0.054	0.5
VAS pain	0.272	<0.0001	0.240	0.0006
VAS Fatigue	0.250	<0.0001	0.330	<0.0001

Log: logarithmically transformed; RA: rheumatoid arthritis; DAS28: Disease Activity Score in 28 joints; HAQ: Health Assessment Questionnaire; N: number; VAS: visual analogue scale.

Results

Participants

A total of 643 patients comprising 441

Table III. Number of patients and depression scores by gender, ethnic grouping, rheumatoid factor status and antirheumatic agent use, in public and private healthcare patients with RA*.

	Public			Private		
Characteristic	number	depression score	*p*-value	number	depression score	*p*-value
Sex						
Women	383	3.5 ± 2.0		162	2.4 ± 1.6	
Men	58	4.3 ± 2.1	0.01	40	1.6 ± 1.8	0.02
Black						
Yes	295	3.6 ± 1.9		9	2.6 ± 1.1	
No	146	3.6 ± 2.3	0.8	193	2.2 ± 1.7	0.4
White						
Yes	60	3.6 ± 2.4		168	2.2 ± 1.6	
No	381	3.6 ± 2.0	0.9	34	2.7 ± 1.8	0.1
Asian						
Yes	49	3.5 ± 2.4		16	2.7 ± 1.6	
No	392	3.6 ± 2.0	0.8	186	2.2 ± 1.7	0.4
Mixed						
Yes	37	3.6 ± 2.1		9	2.2 ± 1.6	
No	404	3.5 ± 2.4	0.9	193	2.7 ± 2.1	0.4
RF positive						
Yes	330	3.6 ± 2.0		166	2.2 ± 1.6	
No	111	3.5 ± 2.1	0.6	36	2.5 ± 1.7	0.3
DMARDs use						
Yes	439			190	2.2 ± 1.6	
No	2	…	…	12	3.5 ± 2.1	0.07
NSAIDs use						
Yes	78	3.6 ± 1.9		44	2.1 ± 1.6	
No	363	3.6 ± 2.1	0.9	158	2.3 ± 1.7	0.4
Prednisone use						
Yes	20	3.2 ± 1.8		14	1.7 ± 2.1	
No	421	3.6 ± 2.1	0.1	188	2.3 ± 1.6	0.3

*Depression scores are expressed as mean ± SD. The association of DMARDs use with depression in public healthcare patients was not assessed since only 2 patients were not employing such agents. RA: rheumatoid arthritis; RF: rheumatoid factor status; DMARDs: disease modifying agents for rheumatic disease; NSAIDs: non-steroidal anti-inflammatory drugs.

Table IV. Multivariable models of the relationship of demographic and RA characteristics with the AIMS depression scores in public and private healthcare patients with RA.

	Public		Private	
Characteristic	Standardised β [95% CI]	*p*-value	Standardised β [95% CI]	*p*-value
Age, years	-0.11 [-0.21, -0.02]	0.02	0.03 [-0.11, 0.17]	0.07
Female sex	-0.13 [-1.22, -0.03]	0.007	0.13 [-0.00, 0.25]	0.06
Ethnic origin				
White	Reference		Reference	
Black	0.03 [-0.07, 0.13]	0.6	-0.03 [-0.18, 0.14]	0.7
Asian	0.02 [-0.09, 0.13]	0.7	0.00 [-0.60, 0.60]	1.0
Mixed ancestry	-0.02 [-0.12, 0.09]	0.7	-0.04 [-0.21, 0.13]	0.6
DAS28	0.00 [-0.10, 0.10]	1.0	0.09 [-0.08, 0.27]	0.3
HAQ score	0.25 [0.15, 0.36]	<0.0001	0.23 [0.05, 0.41]	0.01
N deformed joints	-0.02 [-0.12, 0.09]	0.8	-0.03 [-0.16, 0.11]	0.7
VAS pain	0.16 [0.02, 0.29]	0.02	-0.09 [-0.28, 0.10]	0.3
VAS fatigue	0.04 [-0.09, 0.18]	0.5	0.23 [0.07, 0.39]	0.006
DMARDs use	0.06 [-0.03, 1.49]	0.2	-0.16 [-0.29, 0.03]	0.02

β: regression coefficient; CI: confidence interval; DAS28: Disease Activity Score in 28 joints; HAQ: Health Assessment Questionnaire; N: number; VAS: visual analogue scale; DMARDs: disease-modifying agents.

public and 202 private healthcare cases, were investigated (Table I).

Baseline characteristics in public compared with private healthcare centre patients.

We assessed disparities in the prevalence or extent of baseline recorded characteristics in public compared with private healthcare patients (Table I). Patients seen in the public healthcare center were more frequently female and substantially more often of black and less frequently of white ancestry. Physical disability, the number of deformed joints and visual analogue scale (VAS) pain and fatigue were each larger in public compared with private healthcare patients. Conventional DMARDs were employed 5.4% more often in public than private healthcare patients. On the other hand, biologic therapy use was not only overall infrequent but further restricted to patients that were enrolled in our private healthcare sector. South African public healthcare patients have currently no access to biologic therapy whereas our private sector medical schemes approve the use of biological agents only in patients with substantial RA disease activity that is refractory to multiple conventional DMARDs. The overall infrequent use of oral glucocorticoids by us in the present patient cohorts is a result of our previously reported unfavorable findings with the use of this intervention in RA (28). We treat disease activity exacerbations with intra-articular corticosteroids and concurrent conventional DMARD regimen intensifications in both our public and private healthcare sectors (35, 36).

Depression scores and their associated demographic and RA characteristics in public compared to private healthcare patients with RA

The mean ± SD AIMS depression score was markedly larger in public compared to private healthcare patients (3.6±2.1 *vs*. 2.3±1.7, $p<0.0001$).

Univariate associations of demographic and RA characteristics with AIMS depression scores in public and private healthcare patients are shown in Tables II (continuous variables) and III (categorical variables). In public

Table V. Multivariable model of the relation of recorded characteristics with the AIMS depression score in all 643 patients with RA.

Characteristic	Standardised β [95% CI]	*p*-value
Public healthcare	0.22 [0.12, 0.32]	<0.0001
Age, years	-0.07 [-0.14, 0.00]	0.06
Female sex	-0.05 [-0.12, 0.03]	0.2
Ethnic origin		
White	Reference	
Black	0.01 [-0.06, 0.07]	0.9
Asian	0.02 [-0.09, 0.12]	0.8
Mixed ancestry	-0.03 [-0.13, 0.07]	0.5
DAS28	0.01 [-0.08, 0.09]	0.9
HAQ score	0.25 [0.16, 0.34]	<0.0001
N deformed joints	-0.03 [-0.11, 0.05]	0.5
VAS pain	0.10 [-0.02, 0.19]	0.09
VAS fatigue	0.11 [0.01, 0.21]	0.04
DMARDs use	-0.06 [-0.13, 0.02]	0.1

AIMS: Arthritis Impact Measurement Scales; β: regression coefficient; CI: confidence interval; DAS28: Disease Activity Score in 28 joints; HAQ: Health Assessment Questionnaire; N: number; VAS: visual analogue scale; DMARDs: disease-modifying agents.

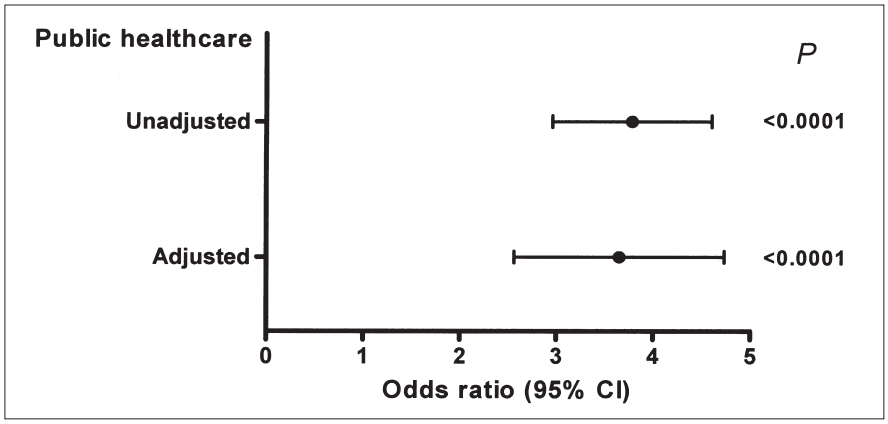

Fig. 1. Association of public healthcare attendance with an Arthritis Impact Measurement Scales depression score of ≥4 in univariate and age, sex, ethnic origin, rheumatoid factor status, disease activity, physical disability, the number of deformed joints, the VAS pain and fatigue and DMARDs use adjusted analysis.

healthcare patients, male sex, disease activity, physical disability, the number of deformed joints and the VAS pain and fatigue were associated with depression scores. In private healthcare patients, female sex, disease activity and the VAS pain and fatigue and non-use of DMARDs (with borderline significance (p=0.07)) related to depression scores. Since only 2 patients in the public healthcare sector were not using DMARDs and biologic therapy was prescribed in only 6 private and no public healthcare patients, associations between depression scores and DMARDS use in public healthcare patients and biologic therapy in public and private healthcare patients, respectively, were not assessed.

Multivariable regression models of the relationship of demographic and RA characteristics with depression scores in public and private healthcare patients are presented in Table IV. In public healthcare patients, older age and female sex independently associated with lower depression scores whereas physical disability and the VAS pain related to higher depression scores. In private healthcare patients, physical disability similarly associated with larger depression scores. However, depression scores in these patients were not related to age, female sex and the VAS pain and associated with a high VAS fatigue and non-DMARDs use. When we added rheumatoid factor status as an independent variable in the regression models in Table IV, the results were not materially altered and rheumatoid factor status was not associated with the AIMS depression score (results not shown).

The independent associations of public healthcare attendance with depressive symptoms and a high AIMS depression score (≥4) in patients with RA

The independent association of public healthcare attendance and demographic and RA characteristics with the AIMS depression score in all patients is shown in Table V. Public healthcare and the HAQ score were most strongly associated with depressive symptoms and with similar standardised regression coefficients (0.22 and 0.25, respectively) and widely overlapping 5–95% confidence intervals. Fatigue was the only remaining variable that related independently to depressive symptoms.

Since our previous analyses had revealed that demographic and RA characteristics and their relationship with depressive symptoms differed by healthcare sector, we further assessed the association of public healthcare attendance with an AIMS depression score of ≥4 in all patients in a logistic regression model in which age, sex, ethnic origin, rheumatoid factor status, disease activity, physical disability, the number of deformed joints, the VAS pain and fatigue and DMARDs use were adjusted for. Public healthcare attendance remained strongly associated with an AIMS depression score of ≥4 (OR [95% CI]=3.33 [1.95, 5.67], p<0.0001). This is illustrated in Figure 1.

Discussion

In the present study, we found for the first time that in a lower-middle income country (23), patients with RA that belong to the developing population experience substantially more depressive symptoms than those that form part of the developed population. Public healthcare patients had more severe RA and the relationship of several demographic and disease characteristics with depressive symptoms differed by healthcare sector. However, adjustment for these characteristics did not alter the association of public healthcare attendance with depressive symptoms. In all patients, public healthcare attendance was as strongly associated with depressive symptoms as was the HAQ score.

Our results document that knowledge on depressive symptoms that derives from high income countries may not apply in the developing world (16, 17, 20-22). In the recent South African Stress and Health Study, a nationally representative household survey in which the World Health Organization Composite Diagnostic Interview was employed, the 12 months burden of depressive symptoms was smaller than in the USA that is a high income country,

but larger than in Nigeria that is a low income country (33). In this context, it is particularly striking that one in two South Africans with RA that belong to the developing population of this country (24, 25, 28) have an AIMS depression score that indicates that they could be assessed as being depressed at interview. Reported findings (16, 17, 19-23) together with our results indicate that the connectedness between RA and depressive symptoms is markedly enhanced in the developing compared to developed population of South Africa. This substantiates the need for routine evaluation of depressive symptoms by physicians that treat patients with RA that belong to developing populations. Although the role of interventions including the use of antidepressants in such settings requires further elucidation, recent evidence reported that depression can be effectively assessed and managed even in low-resource healthcare settings (17, 19, 23, 37, 38). Women are at higher risk for depression than men (4), particularly in developing countries (19, 39) including in South Africa (32). Gender and age were not significantly related to depressive symptoms in our private healthcare patients. In contrast, in our public healthcare sector, men and young patients experienced more depressive symptoms. Pain and fatigue are markers of distress in patients with RA that live in developed countries (5, 40, 41). Fatigue was associated with depressive symptoms only in our private healthcare patients and pain only in our public healthcare patients. Notably in the present context, Goulia and colleagues recently reported that pain plays a different role in disease outcome in younger compared to older patients with rheumatic diseases including RA (42). However, age did not differ by healthcare sector in the present study. Relevant non-use of DMARDs was only recorded in our private healthcare patients. This was almost invariably due to patient perceived inadequate financial support by private sector medical schemes (data not shown) and was associated with increased depressive symptoms, a finding that was reported in RA studies performed in developed countries (4, 5). In contrast to reported findings in high income countries (6, 43, 44), ethnic origin was not independently related to depressive symptoms in South African patients with RA.

The present study has several limitations. Our cross-sectional design precludes drawing inferences on the direction of causality. Additionally, bidirectional relationships between RA characteristics including pain and depressive symptoms in patients with RA in developed countries were reported (12). Therefore, our current findings require elucidation in longitudinal and interventional investigations. As applies to almost all studies on depressive symptoms in RA (3), we used a self report assessment and not a clinical interviews report (14). Self report assessment can inflate depression rates due to somatic items in the scale (45). Such characteristics are not included in the AIMS depression score. The AIMS depression score, however, has not been validated in the South African population at large, and since factor invariance across ethnic groups has been supported (33), our findings should be interpreted with caution. Selection bias based on different resource utilisation and availability in our private compared to public healthcare sector also needs to be considered. However, with the exception of access to biologic therapy and as a consequence of our previously reported findings (29), the monitoring and management strategies are currently similar in our public and private health sector. Comorbidities as well as a number of social factors that are reportedly major determinants of mental disorders in low and middle income countries, were not systematically assessed. The latter include marital status, low education, poverty, unemployment, social exclusion, disrupted family background, interpersonal relationships and conflict (19, 34, 46). The role of comorbidities and social factors as well as of their interaction with disease characteristics in the causation of depressive symptoms in patients with RA that belong to developing populations require further investigation. In fact, reported evidence further indicates that social factors could account for the enhanced RA severity in our public healthcare patients (9). Additionally, it is possible that RA patients that belong to developing populations are less likely to seek medical care when they have less severe disease. However, enhanced RA severity did not explain the association of public healthcare attendance with depressive symptoms in the present investigation. Our patients were selected on the basis that they had RA. The current findings should therefore not be extrapolated to individuals with other medical illnesses. Compared to their private counterparts, our public healthcare patients with RA are inherently sociodemographically disadvantaged (27, 28). In the developed world, this characteristic reportedly can reduce the number of patients that are compliant with medication and recommended clinic visits (4, 5, 20). Such patients would be expected to have even higher rates and severity of mental illness (20). Thus, reduced compliance with recommended clinic visits would be expected to have rendered conservative estimates of the burden of depressive symptoms in public healthcare patients with RA (20). Further, our findings on non-DMARD use in private compared to public healthcare patients argue against non-compliance with medication in our public healthcare sector. Finally, non-caucasian groups were small in our private healthcare sector patients. When the data were analysed after inclusion of all non-caucasians in one group (n=34 in private healthcare), the findings remained unaltered (data not shown).

In conclusion, the present study that was performed in a lower-middle income country, revealed a markedly enhanced burden of depressive symptoms in patients with RA that formed part of its developing population, independent of age, sex, ethnic origin and disease characteristics. Factors that account for the enhanced connectedness between RA and depressive symptoms in developing populations need further exploration. Such undertaking may be essential in delineating optimal interventions that are also likely to require inclusion of government policy makers (17, 18, 23, 37). In the meantime, since the avail-

ability of only limited resources does not preclude the provision of effective management of depression (17, 18, 23, 37, 38), our results indicate that depressive symptoms should be routinely addressed in patients with RA that form part of developing populations.

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