Makara Journal of Health Research

Volume 23
Issue 3 *December*

Article 9

12-1-2019

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Kek Xin Yi
*Bintulu Hospital, 97000 Bintulu, Sarawak, Malaysia*, kek_forest@hotmail.com

Asrul A. Wahab
*Universiti Kebangsaan Malaysia, Malaysia*, saw@ppukm.ukm.edu.my

Mohamad Norazrin Mohd Abas
*Miri Hospital, 98000 Miri, Sarawak, Malaysia*, azrin.abas@gmail.com

Siti Amira Kamarul Abdul Wahid
*Women and Child Hospital Sabah, 88996 Kota Kinabalu, Sabah, Malaysia*, drmirakama@gmail.com

Faridah Hanun Othman
*Balik Pulau Hospital, 11500 Balik Pulau, Penang, Malaysia*, hanunothman@yahoo.co.uk

*See next page for additional authors*



## Recommended Citation

Yi KX, Wahab AA, Abas MNM, Wahid SAK, Othman FH, Said MSM. Correlation between carotid intima-media thickness and serum TNF-a levels in female with rheumatoid arthritis of different ethnicities: A single-centre experience in Malaysia. Makara J Health Res. 2019;3(3):176–180.

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## Authors

Kek Xin Yi, Asrul A. Wahab, Mohamad Norazrin Mohd Abas, Siti Amira Kamarul Abdul Wahid, Faridah Hanun Othman, and Mohd. Shahrir Mohamed Said

Makara J Health Res. 2019;23(3):176–180
doi: 10.7454/msk.v23i3.1145

# Correlation between carotid intima-media thickness and serum TNF-α levels in female with rheumatoid arthritis of different ethnicities: A single-centre experience in Malaysia

Kek Xin Yi[1], Asrul Abdul Wahab[2*], Mohamad Norazrin Mohd Abas[3], Siti Amira Kamarul Abdul Wahid[4], Faridah Hanun Othman[5], Mohd Shahrir Mohamed Said[6]

1. Bintulu Hospital, 97000 Bintulu, Sarawak, Malaysia
2. Department of Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia
3. Miri Hospital, 98000 Miri, Sarawak, Malaysia
4. Women and Child Hospital Sabah, 88996 Kota Kinabalu, Sabah, Malaysia
5. Balik Pulau Hospital, 11500 Balik Pulau, Penang, Malaysia
6. Department of Internal Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia, 56000 Cheras, Kuala Lumpur, Malaysia

*E-mail: saw@ppukm.ukm.edu.my*

## Abstract

**Background**: Rheumatoid arthritis (RA) is a destructive chronic inflammatory disease with intra- and extra-articular manifestations. It is associated with accelerated formation of arteriosclerosis. The aim of this study was to ascertain the correlation between endothelial dysfunction using carotid intima–media thickness (cIMT) and tumor necrosis factor-alpha (TNF-α) levels among the three main ethnic groups in Malaysia: Malay, Chinese, and Indian. **Methods**: This cross-sectional study included 23 females with RA belonging to three different ethnic groups who were free from cardiovascular risk factors. Blood sample was taken from each patient to measure serum TNF-α level, C-reactive protein (CRP), and erythrocyte sedimentation rate (ESR). Carotid ultrasonography was performed for cIMT analysis. The Disease Activity Score (DAS)-28 scoring system was used to assess disease activity. **Results**: Patients' median age and disease duration were 47 and 5 years, respectively. No thickened cIMT was found in any of the patients. The duration of disease, TNF-α level, cIMT, DAS-28 CRP, and DAS-28 ESR showed no significant correlations in the different ethnic groups ($p > 0.05$). **Conclusion:** This study indicates that cIMT and serum TNF-α levels among females with RA in Malaysia may not be affected by ethnicity.

*Keywords: carotid intima–media thickness, ethnic groups, rheumatoid arthritis, tumor necrosis factor-alpha*

## Introduction

Rheumatoid arthritis (RA) is a destructive chronic inflammatory disease that mainly affects the joints but may also have extra-articular manifestations. Prevalence of RA in the low- and middle-income in six of World Health Organization's regions showed the highest prevalence was in America with 1.25% and the lowest was in Eastern Mediterranean with 0.37%, with the prevalence in Southeast Asia was 0.4%.[1] In Malaysia, the female–to–male ratio of people affected by RA is 8:1, and the ethnic group mainly affected by the disease is Indian.[2] The production of proinflammatory cytokines, such as tumor necrosis factor (TNF), interleukins (IL) such as IL-1, and IL-6, contributes greatly to the inflammatory process. Tumor necrosis factor can induce the production of other proinflammatory cytokines (IL-1 and IL-6) and the release of chemokines that attract leukocytes, which eventually cause the destruction of articular cartilage and subchondral bone by initiating the action of proteolytic enzymes in the disease.[3] Tumor necrosis factor and IL-6 play important role in innate immune response. It was shown that TNF involved in leukocyte activation, adhesion and migration, endothelial activation and angiogenesis and some other factors that related to endothelial function and repair.[4] Thus, TNF-α plays an important role in endothelial dysfunction in patients with RA.[5]

Rheumatoid arthritis has been reported to be associated with increased risk of cardiovascular disease with approximately 50% of premature death in RA was due to cardiovascular disease.[6] The associations among endothelial dysfunction, atherosclerosis, and cardiac events are well established. Intima–media thickness of the common carotid artery can predict cardiovascular

events. Ultrasonographic measurement of carotid intima–media thickness (cIMT) has been described as a sensitive, noninvasive, and reproducible method for identifying and quantifying subclinical vascular disease and for assessing cardiovascular disease risk. As confirmed by many studies, cIMT is thicker in RA patients than in healthy controls.[7–9] A previous study showed that cIMT was thicker in RA population and this was influenced by region, race, age, body mass index (BMI) and disease duration.[7] It was also shown that the cIMT was significantly by African geographically and non-Caucasion ethnically.[7] However, this could not be generalized to multicultural and different racial distribution in Malaysia. Thus, the current study has been conducted to measure the correlation between cIMT, level of TNF-α, and Disease Activity Score (DAS)-28 among females with RA from the major ethnic groups in Malaysia.

## Methods

**Patients.** This cross-sectional study screened 160 consecutive RA patients who attended our Rheumatology Clinic in the Hospital Canselor Tuanku Muhriz, Universiti Kebangsaan Malaysia Medical Centre within the study period. After screening, only 23 females with RA aged 18 years and above were included in this study. All participants provided written informed consent. We excluded from the study pregnant women, patients with co-morbidities of diabetes mellitus, hypertension, other connective tissue diseases, acute infection, and obesity, and patients taking biological agents, such as etanercept. In defining obesity in our study population, we followed the Malaysia Clinical Practice Guidelines on Management of Obesity 2004.[10] The diagnosis of RA was based on the 2010 ACR-EULAR rheumatoid arthritis classification criteria.[11] The study was approved by the Research and Ethic Committee of UKM Medical Centre, and given the research number FF-2014-061.

**Disease activity assessment.** Disease activity in all patients was evaluated using the DAS-28 criteria. The calculator for DAS-28 was obtained from http://www.4s-dawn.com/DAS28/DAS28.html.[12]

**Laboratory analysis.** Blood samples from the patients were tested in the laboratory for C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and serum TNF-α level. The levels of >0.5 mg/dL and >20 mm/h were considered high for CRP and ESR, respectively. The TNF-α level was measured via the ELISA method using the commercial kit Quantikine® ELISA by R&D System, Minneapolis USA. The test was performed according to the standard ELISA protocol, as included in the manufacturer's protocol. The TNF-α level was measured in pg/mL. The samples were performed in duplicate, and the mean value was taken as the level for each patient's sample.

**Assessment of the cIMT.** The cIMT was measured using ultrasound. In this study, the vessel selected for this analysis was the common carotid artery. The measurement was performed in accordance with the American Society of Echocardio-graphy 2008 Consensus Statement guidelines for cIMT measurement.[13] The ultrasound procedure was performed by a single rheumatologist with more than 10 years' experience in rheumatology.

**Statistical Analysis.** Data were described as counts and percentages if they were categorical. Mean and standard deviation or median were used for continuous data. We carried out multivariate analysis to assess the correlation between different ethnic groups in respect of levels of TNF-α, the DAS-28 score, and cIMT. For the statistical analysis, the two-tailed $p < 0.05$ was considered as significant.

## Results

**Characteristic of patients with RA.** The median age of the females with RA recruited for the study was 47 years. Their ages ranged from 31 to 78 years, with a mean of 49.87 years. Most of the participants were Malays (46.7%), followed by Chinese (33.3%), and Indians (20%). The mean body mass index (BMI) was 23.1. Most participants had had RA for more than two years (N = 17, 73.9%), with a median disease duration of 5 years.

Disease activity was assessed in 28 joints via the DAS, using the ESR and CRP. DAS-28 ESR assessment showed that 20 patients were in the active stage, whereas DAS-28 CRP assessment showed that only 16 of them were actually in the active stage. The results are presented in Table 1.

**cIMT measurement and TNF-α level.** A total of 16 patients had their cIMT measured; the remaining seven patients did not, for various reasons, and they were analyzed as missing values. Examination of carotid arteries showed that carotid artery thickness was normal in all patients, but one patient had carotid plaque. The mean cIMT measurement was 0.089 ± 0.077 mm.

Serum TNF-α level was undetectable in seven patients. The serum TNF-α level ranged from 0 to 31.83 pg/mL. Spearman's rho correlation revealed no significant correlation between the serum TNF-α level and cIMT ($p = 0.389$). Further analysis using the Mann-Whitney U test showed that there was no significant correlation between serum TNF-α level and cIMT, with DAS-28 CRP and DAS-28 ESR having $p > 0.05$.

**Table 1.** Characteristics of patient with rheumatoid arthritis (N = 23)

| Variables | Patients with rheumatoid arthritis |
|---|---|
| Age (years) | 47.00 (41.00, 57.00)** |
| Race | |
| Malay | 10 (43.5%) |
| Chinese | 8 (34.8%) |
| Indian | 5 (21.7%) |
| BMI (kg/m$^2$) | 23.1±3.12* |
| ESR (mm/h) | 48.00 (25.00, 74.00)** |
| CRP (mg/dl) | 0.7 (0.16, 1.84)** |
| Duration of RA (years) | 5.00 (1.00, 9.00)** |
| DAS-28-CRP | |
| Remission (<2.6) | 7 (30.4%) |
| Active (≥2.6) | 16 (69.6%) |
| DAS-28-ESR | |
| Remission (<2.6) | 3 (13%) |
| Active (≥2.6) | 20 (87%) |

*Normally distributed data are presented in Mean ± SD (standard deviation); **Non-normally distributed data are presented in Median (25$^{th}$, 75$^{th}$ centiles); BMI = Body Mass Index; ESR = Erythrocyte Sedimentation Rate; CRP = C-reactive Protein; DAS28-CRP = Disease Activity Score 28-C-reactive protein; DAS28-ESR = Disease Activity Score 28-erythrocyte sedimentation rate.

**Table 2.** Correlation among the duration of disease, TNF-α level, cIMT measurement, DAS-28 CRP, and DAS-28 ESR in different ethnic groups.

| Variables | Median | *p* * |
|---|---|---|
| Duration of disease (years) | | |
| Malay | 7.0 | 0.904 |
| Chinese | 8.5 | |
| India | 9.0 | |
| TNF- α levels (pg/mL) | | |
| Malay | 8.3 | 0.651 |
| Chinese | 5.3 | |
| India | 5.2 | |
| cIMT (mm) | | |
| Malay | 0.4 | 0.275 |
| Chinese | 0.4 | |
| India | 0.2 | |
| DAS28-CRP score | | |
| Malay | 8.0 | 0.251 |
| Chinese | 3.5 | |
| India | 6.0 | |
| DAS28-ESR score | | |
| Malay | 8.0 | 0.415 |
| Chinese | 7.5 | |
| India | 7.3 | |

*****Kruskal-Wallis; TNF- α = Tumor Necrosis Factor-α; cIMT = Carotid Intima Media Thickness; DAS-28-CRP = Disease Activity Score 28-C-reactive protein; DAS-28-ESR = Disease Activity Score -28 Erythrocyte Sedimentation Rate.

The duration of disease did not differ significantly between ethnic groups, and neither did the levels of TNF-α, cIMT, and DAS-28 CRP and DAS-28 ESR, with $p > 0.05$. The analysis is shown in Table 2.

## Discussion

We attempted to examine and compare the relationship between endothelial dysfunction and TNF-α levels in females with RA from the three main ethnic groups in Malaysia. To our knowledge, this is the first such study conducted in Malaysia. We found no significant correlation between cIMT, TNF-α level, and DAS-28 CRP and DAS-28 ESR in females with RA from the three main ethnic groups in Malaysia.

We note that, due to small sample size, the patients included in this study might not be a true representation of the females with RA in Peninsular Malaysia. However, the mean age of the participants (49.87 years) in this current study was almost similar to that in a previous study of females with RA (49.30 years).[2] Previous studies have demonstrated that cIMT in patients with RA is significantly larger than in patients without RA.[7–9] In this study, all the patients who underwent cIMT assessment had normal cIMT (0.089 ± 0.077 mm), but one patient had carotid plaque.

TNF-α levels have been reported to be higher in RA patients, particularly those in whom the disease is active.[14] In this study, the TNF-α serum level ranged from 0 to 31.83 pg/mL. We have been unable to conclude if this level is higher than is that in the healthy population because there was no healthy control included. However, there was no significant correlation found between serum TNF-α levels and cIMT with disease activity. A similar result was obtained in a previous study, in which no correlation was found between the cIMT value and the parameters of RA activity and disease duration; however, TNF-α was not measured.[15] Another study that evaluated the coronary artery calcium (CAC) score as a marker for coronary plaque showed no significant association between CAC score and inflammory markers namely C-reactive protein, fibrinogen and IL-6.[16] However, the role of TNF-α in the development of endothelial dysfunction may also be directly shown by the significant improvement of endothelial function in those treated with anti- TNF-α.[17]

We found no significant difference in disease duration among RA patients from the three main races in Malaysia. We also showed, via the DAS-28 score, that there was no significant difference between people of different ethnicities. A study has shown that white people with RA in the United States had less disease activity and better function compared with non-white people when DAS-28 scores were used as assessment tools.[18] Another study in United States and Canada had reported that Hispanics and African Americans performed worse in disability index, pain and global health.[19] TNF-α levels in Asian and Caucasians patients with RA have been noted to be higher than are those in controls.[20] However, no previous study has compared the differences in the TNF-α levels in RA patients of different ethnicities in Malaysia. In terms of cIMT, we found no significance difference between patients of different ethnicities. Previous study has shown that ethnicity may play a role in the endothelial dysfunction in RA populations.[21]

We acknowledge that this study has some limitations. First, as it was conducted in a single health-care center in Malaysia, the findings cannot either be generalized or represent either the true epidemiology or scenario of endothelial dysfunction among females with RA in Malaysia. Second, the number of samples was small; however, this was due to our stringent inclusion criteria. Most of the females with RA we screened had co-morbidities that excluded them participating in this research.

## Conclusion

Based on the current communication, ethnicity in Peninsular Malaysia may not affect disease activity and the development of endothelial dysfunction among females with RA. However, the small number of the patients included in this study does not warrant a concrete conclusion. To confirm our findings, further study with a larger sample size and multicenter involvement in Malaysia is required.

## Acknowledgments

The authors express their sincere thanks to the Dean of Faculty of Medicine, Universiti Kebangsaan Malaysia, for his support to conduct this research.

## Funding

This research had been funded by the Faculty of Medicine, Universiti Kebangsaan Malaysia under Fundamental Grant with research code FF-2014-061.

## Conflict of Interest Statement

The authors declare no conflict of interest.

**Received: July 2nd, 2019 Accepted: October 23rd, 2019**

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