

**Original article:**

**ANTINUCLEAR ANTIBODIES IN PRIMARY OSTEOARTHRITIS OF THE KNEE: A CASE-CONTROL STUDY**

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**ABSTRACT**

**Objective:** Although osteoarthritis (OA) is widely accepted as a degenerative disease, auto-immune processes are believed to be involved in the pathogenesis. There are limited studies in this area and most of them focused on antibodies against chondrocyte membrane. In an attempt to address the paucity of evidence in this regard, we explored the clinical significance of antinuclear antibody (ANA) in primary osteoarthritis of the knee (OAK).

**Method:** We studied 106 patients with primary osteoarthritis of at least 1 knee and 63 healthy controls from two tertiary centres in Malaysia from September 2005 to May 2012. All subjects were tested for ANA by immunofluorescence testing, and a titer of 1:40 and above was considered positive. Besides, the radiographs of bilateral knees were evaluated for grading, tibiofemoral compartment involvement and total knee replacement (TKR) implants. We compared the clinical characteristics between the ANA positive and ANA negative OAK cases.

**Results:** The incidence of ANA positivity among the cases (39.4 %) was higher than the controls (27 %) but this difference was statistically insignificant ( $p=0.754$ ). ANA positive cases showed significantly higher incidence of bilateral and Grade IV OAK with higher frequency of TKR. In the multiple regression analysis, bilateral OAK ( $p<0.0001$ ; odds ratio 9.00), Grade IV OAK ( $p<0.001$ , odds ratio 3.44) and TKR ( $p=0.009$ ; odds ratio 2.97) remained associated with ANA positivity.

**Conclusions:** ANA test is a potential prognostic tool in primary OAK and its positivity is associated with the clinical outcomes of bilateral, Grade IV OAK and TKR.

**Keywords:** antinuclear antibodies, primary osteoarthritis of the knee, osteoarthritis

**INTRODUCTION**

The burden of osteoarthritis (OA) is on the rise with the steadily expanding aging population worldwide. It is disappointing that the treatment of this condition has not evolved much since its discovery centuries ago. Exploring the underlying mechanisms

involved is of paramount importance to develop novel therapies. Although OA is well established as a degenerative disease mainly caused by mechanical stresses and aging factors, the pathogenesis is probably more complex than previously thought. It was not until the 1980s that the concept of autoimmunity in OA emerged (Cooke et al., 1980;

Jasin, 1985). From a research perspective, autoimmunity in OA has not received the attention it deserves. Hence, the available evidence on autoantibodies in OA are still far from being conclusive.

Studies in the past on OA have reported humoral and cellular immune responses to cartilage-related components such as YKL-39, cartilage intermediate layer protein, and osteopontin (Yuan et al., 2003; Mollenhauer et al., 1988; Tsuruha et al., 2002; Sakata et al., 2001). In fact, anti fibulin-4 directed against fibulin-4, which is an extracellular matrix protein was found to be more prevalent in OA than rheumatoid arthritis (RA) (Xiang et al., 2006). The limited studies related to autoimmunity in OA have mostly looked into antibodies against chondrocyte membrane (Jasin, 1985; Mollenhauer et al., 1988; Tsuruha et al., 2002; Sakata et al., 2001).

Antinuclear antibody (ANA) is one of the most commonly performed screening tests in the detection of autoimmune diseases. Although strongly associated with Systemic Lupus Erythematosus, it is not highly specific for this condition. ANA is found in a wide array of autoimmune diseases encompassing connective tissue diseases like RA and non rheumatic diseases such as primary biliary cirrhosis, Graves disease and autoimmune hepatitis (Colglazier and Sutej, 2005).

A few studies in the past have examined the incidence of ANA positivity among OA patients (Steven et al., 1984; Bonroy et al., 2012; Gaddy et al., 2012). Although Steven et al. (1984) reported that 15 % of OA patients tested ANA positive, the role of ANA in this condition was not further explored. In 2009, Udartsev et al. discovered that in osteoarthrosis the frequency of chromosomal aberrations correlated positively with the amount of ANA in the synoviocytes. However, till today there is still lack of published data on ANA in OA and hence, the clinical significance of ANA in this regard remains largely undetermined. We hypothesize that ANA positivity could be a factor influencing the severity of OA. The

basis for this hypothesis is the well documented association between circulating autoantibodies and more aggressive course of disease in certain conditions such as rheumatoid arthritis and primary biliary cirrhosis. Autoantibodies have been postulated to trigger immune mediators of organ damage (Aletaha et al., 2012; Edelman and Russell 1983; Rigopoulou et al., 2005). Hence, the aim of this study was to compare the clinical characteristics of the ANA-positive with the ANA-negative OA of the knee patients.

## METHODS

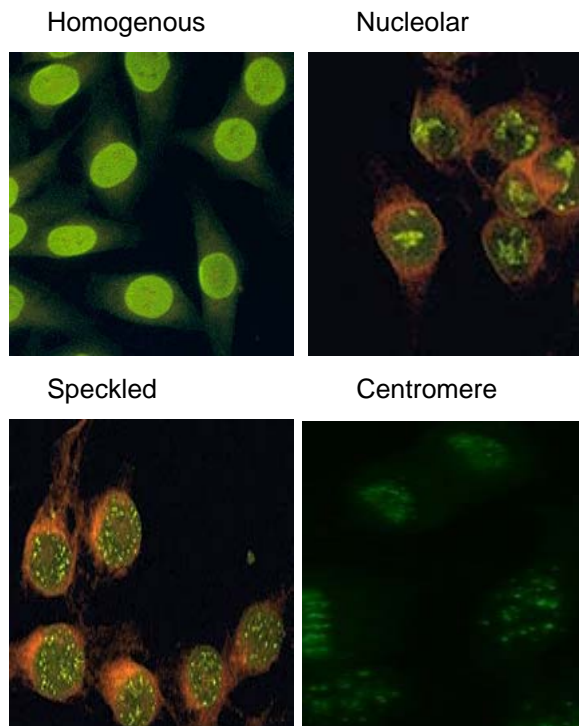
### *Patients*

We studied 106 patients with primary osteoarthritis of at least 1 knee (the cases) and 63 healthy controls from two tertiary centres in Malaysia i.e Universiti Kebangsaan Malaysia Medical Centre and Putrajaya Hospital from September 2005 to May 2012. The study was conducted in accordance with the ethical rules and policies of both the centres. Patients enrolled in this study were evaluated by rheumatologists and/or orthopaedic surgeons. The diagnosis of primary osteoarthritis of the knee (OAK) was established based on history, physical examination and radiographic findings of OAK. The criteria to be classified as OAK included knee pain, osteophytes and at least 1 of the following 3; age >50 years, early morning stiffness of <30 minutes duration or crepitus (Altman et al., 1986). The radiographs of the knees were assessed and graded according to the Modified Kellgren Lawrence grade (Pettersson et al., 1997). The grade for the most severe tibiofemoral compartment was recorded. Besides, the radiographs were reviewed for total knee replacement (TKR) implants, in addition to the history of TKR from the cases.

All controls had no clinical history, physical examination or radiographic findings of OAK. The medical records of all cases and controls were reviewed. Those with documented autoimmune disorder were excluded from this study.

### ANA assay

All subjects were tested for antinuclear antibodies (ANA). The ANA was detected by indirect immunofluorescence testing by HEp-2 human epithelial cells, and a titer of 1:40 and above was considered positive. Multispot slides with fixed HEp-2 cells were incubated with diluted sera for 30 min at room temperature. Figure 1 shows the micrographs of the immunofluorescence patterns identified. To ensure that both the cases and controls had no undiagnosed autoimmune disorder that could influence the results, serum was checked for rheumatoid factor, anti-smooth muscle, anti-mitochondrial, antidouble stranded DNA, extractable nuclear antibodies, lupus anticoagulant and anticardiolipin antibodies among subjects who tested ANA positive. One control had positive antidouble stranded DNA and 5 cases had positive extractable nuclear antibodies and were excluded from the statistical analysis.



**Figure 1:** Micrographs of the immunofluorescence staining patterns. HEp-2 cells were incubated with diluted sera for 30 min at room temperature. Representative staining patterns are shown.

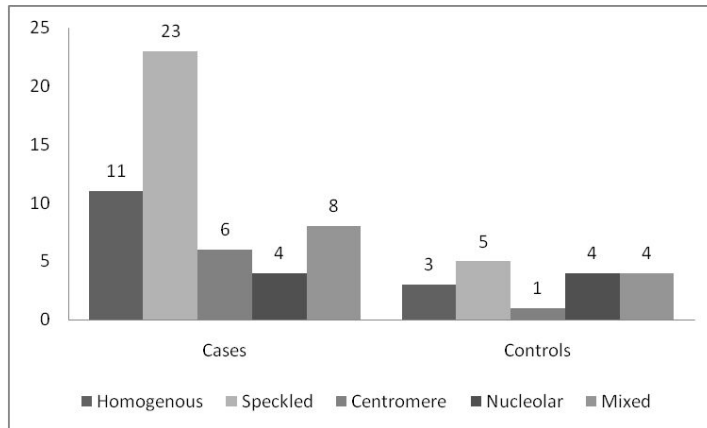
### Statistical analysis

Data analysis was performed using the SPSS 17.0 software for Windows. Data are shown as number of cases, percentages, mean  $\pm$  SD and median (range). We used the Fisher's exact probability test and Chi square test for comparison of categorical variables whereas the student T test and Mann-Whitney U test for comparison of mean and median values respectively. Binary logistic regression analysis was performed to identify the parameters which were significantly associated with the ANA. A p value of  $<0.05$  was considered statistically significant.

## RESULTS

### ANA positivity

The baseline characteristics of the 132 cases and 63 controls studied are shown in Table 1. More than half of the cases and controls were females. The incidence of ANA positivity among the cases (39.4 %) was higher than the controls (27 %). This difference, although interesting, was not statistically significant ( $p=0.754$ ). Similarly, although the median ANA titre was higher among the cases (1:320), the difference was not of statistical significance ( $p=0.550$ ). There were 5 immunofluorescence patterns detected i.e speckled, homogenous, nucleolar, centromere and mixed pattern (combination of 2 or 3 of the earlier patterns). Intriguingly, among the ANA positive subjects, the most commonly observed immunofluorescence staining pattern was speckled for both cases and controls. Almost half (44.2 %) of ANA positive OAK patients exhibited the speckled pattern (Figure 2).



**Figure 2:** The immunofluorescence staining pattern among cases and controls. Figures represent the numbers of positive cases in each category.

**Table 1:** Demographic data of the study population

Characteristic	Cases n=132 (%)	Controls n=63 (%)	p value
Mean age, years (SD)	62.51 ± 9.17*	60.37 ± 8.21*	0.117
Race			
Malay	83 (62.9)	40 (63.5)	0.152
Chinese	25 (18.9)	12 (19.0)	
Indian	24 (18.2)	11 (17.5)	
Gender			
Male	41 (31.1)	12 (19.0)	0.087
Female	91 (68.9)	51 (81.0)	
ANA positivity	52 (39.4)	17 (27.0)	0.754
Median ANA titre (range)	1:320 (1:40-640)**	1:160 (1:40-640)**	0.550

\*Data expressed as mean ± standard deviation, \*\*Data expressed as median (range)

**Table 2:** Comparison between the ANA positive group and ANA negative group of patients with primary osteoarthritis of the knee

Characteristic	ANA positive n=52 (5)	ANA negative n=80 (%)	p value
Mean time since osteoarthritis diagnosis, years (SD)	5.63 ± 2.42*	6.31 ± 3.01*	0.174
Mean BMI, kg/m <sup>2</sup> (SD)	29.12 ± 4.81*	30.33 ± 4.74*	0.157
Bilateral involvement of the knees	36 (69.2)	16 (20.0)	<b>&lt;0.001</b>
Modified Kellgren–Lawrence grade in most severe tibiofemoral compartment			
Grade II	9	20	<b>0.003</b>
Grade III	12	36	
Grade IV	31	24	
Treatment			
Supportive therapy	33 (63.0)	67 (84.0)	<b>0.012</b>
Total knee replacement	19 (36.5)	13 (16.3)	
Unilateral	13	11	
Bilateral	6	2	
Tibiofemoral compartment with more severe features of osteoarthritis			
Medial	39 (75.0)	61 (76.3)	1.000
Lateral	13 (25.0)	19 (23.8)	

\*Data expressed as mean ± standard deviation

**Table 3:** Regression analysis of the relationship of ANA positivity with bilateral, severe knee involvement and the frequency of TKR

	Odds ratio	95 % confidence interval	p value
Bilateral OAK	9.000	4.026 to 20.117	< 0.001
Grade IV OAK	3.444	1.657 to 7.160	< 0.001
TKR	2.967	1.308 to 6.734	0.009

**Effects of ANA positive status on knee involvement and the frequency of total knee replacement (TKR) among OAK patients**

Table 2 outlines the differences between the ANA positive and ANA negative cases. According to the World Health Organization classification, the mean BMI of the ANA positive patients (29.12 kg/m<sup>2</sup>) fell under the pre-obese category (25.00-29.99 kg/m<sup>2</sup>) whereas the ANA negative group (30.33) was in the Obese Class I category (30.00-34.99) (WHO Expert Consultation, 2004)). However, the difference in the mean BMI between both the groups was statistically insignificant (p=0.157). Despite the lower BMI, the ANA positive OAK patients showed significantly higher incidence of bilateral disease with higher grade of OAK and higher frequency of TKR with p values of <0.001, 0.003 and 0.012 respectively. In the multiple regression analysis model, bilateral OAK (p< 0.0001; odds ratio 9.00), Grade IV OAK (p<0.001, odds ratio 3.44) and TKR (p=0.009; odds ratio 2.97) remained associated with ANA positivity (Table 3).

**DISCUSSION**

The concept of autoimmunity in OA is barely a few decades old. The pathophysiology of OA does involve cytokines and growth factors but probably to a lesser extent than in RA. It has been postulated that interleukin 1 and tumour necrosis factor-beta activate enzymes that accelerate proteolytic digestion of cartilage. Growth factors such as tissue growth factor-beta and insulin growth factor-1 are believed to interfere with the physiological processes of cartilage synthesis and repair. An imbalance between cartilage synthesis and catabolism with more of the latter results in OA

(Creamer and Hochberg, 1997; Manek and Lane, 2000). More recently, Scanzello et al. (2008) describe OA as a chronic wound with the activation of Toll-like receptors and their signaling pathways. To date, there are less than 40 human studies focusing on the autoimmunity aspect of OA, based on our literature search. This study, therefore, attempted to address the paucity of research in this area by exploring the significance of ANA among patients with OAK. ANA test is a simple immunological investigation which is readily available to clinicians worldwide.

The incidence of ANA positivity in this study among the cases and controls were higher than that of the general population which is reported to be between 5 to 15 % (Mariz et al., 2011; Azizah et al., 1996; Forslid et al., 1994). A possible explanation for this is the higher mean age of this study population which is in the 6<sup>th</sup> decade of life. The prevalence of ANA positivity among healthy people increases with age (Oyeyinka et al., 1995; Xavier et al., 1995). The autoantibodies detected in the controls could be the expression of age-related damaged tissue process rather than subclinical autoimmunity, as suggested by previous data concerning aging process and circulating antibodies (Stone, 1990; Candore et al., 1997).

The novel findings of this study are the associations of ANA positivity with higher occurrence of bilateral primary OAK, Grade IV OAK and rate of TKR. The exact mechanism for these links is a mystery which will hopefully be unlocked by detailed research in years to come. It is tempting to speculate that ANA positivity in OAK is associated with higher levels of proinflammatory cytokines (IL-1beta, IL-6,

IL-12, IL-18 and TNF-alpha) owing to T cell activation based on knowledge derived from studies on ANA in other diseases such as depressive disorder, hepatitis C and sub-acute cutaneous lupus erythematosus (Maes et al., 1991; Atta et al., 2010; Maczynska et al., 2006). As a consequence of the aforementioned, the ANA positive OAK patients probably undergo more accelerated joint damage compared to their ANA negative counterparts.

The speckled pattern of immunofluorescence staining occurred at highest frequency among cases and controls. This result echoes the finding of other studies which have concluded that ANA immunofluorescent pattern lack specificity (Malleon et al., 1997; Wang et al., 1984). The ANA titre was not very useful either in distinguishing OAK patients from the healthy controls. High ANA titres of equal to or more than 1:640 have a positive predictive value for an autoimmune disease (Malleon et al., 2010). These similarities between the cases and controls remind us that although autoimmunity may contribute to the pathogenesis of primary OAK, OA still does not qualify to be labelled as an autoimmune disease.

In conclusion, this case-controlled study suggests that ANA test is a potential prognostic tool in primary OAK. ANA positivity is associated with less favorable disease outcomes such as bilateral, Grade IV OAK and the need for TKR. Nevertheless, this discovery merits further research with preferably large prospective cohort studies.

#### ***Authors' contributions***

Dr. Sakthiswary designed the study and composed this paper. Dr. Norazman was involved in statistical analysis. Dr. Rajalingam and Dr. Hussein were involved in data collection.

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