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ORIGINAL ARTICLE

Diagnostic Value of Anti-Mutated Citrullinated Vimentin versus Anti-Keratin Anti-bodies in early Diagnosis of Rheumatoid Arthritis

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RA,
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VAS

ABSTRACT

Background*: Rheumatoid arthritis (RA) is a chronic autoimmune disease that is associated with many auto-antibodies such as rheumatoid factor (RF), Antiperinuclear factor (APF), anti-citrullinated peptides and antikeratin antibodies (AKA).* ***Objectives:*** *This study aimed to measure the serum level of anti- mutated citrullinated vimentin antibodies (anti-MCV) and detection of AKA in serum of RA patients. Also, for comparison between the results of anti-MCV and AKA in diagnosis of early RA and their correlation with disease activity. Methodology: This study included 30 patients with RA, 30 patients with other rheumatic diseases and 30 matching healthy controls. Patient's assessment measures involved the disease activity score (DAS-28) and visual analogue scale (VAS). Serum samples were collected from patients and controls for measurement of anti-MCV and detection of AKA using enzyme linked immunosorbant assay (ELISA) and indirect immunofluorescent (IIF)) technique respectively.* ***Results:*** *anti-MCV and AKA were significantly higher in patients compared to controls ($p<0.001$). Serum levels of anti-MCV show insignificant correlation with age and sex. While, it correlates significantly with disease duration, erythrocyte sedimentation rate(ESR), DAS28 and VAS ($p<0.001$). The sensitivity and specificity of anti-MCV test in RA patients at disease duration ≤2 years were 84.2 % and 86 % respectively. AKA show insignificant correlation with age, sex, disease duration, ESR but correlated significantly with DAS28 and VAS ($p=0.005$).The sensitivity and specificity of AKA test in RA patients of disease duration ≤ 2 years were 68.4 % and 68.3% respectively.* ***Conclusion:*** *Anti-MCV and AKA have a role in the pathogenesis of RA, the serum level of anti-MCV antibodies was significantly higher in RA patients than in healthy controls. Anti-MCV and AKA could be a useful marker for early diagnosis and follow up of RA patients, they also reflect both activity and severity of RA. Anti-MCV antibody ELISA test was more sensitive and specific than AKA IIF test, it is a quantitative test while AKA IIF is a screening test and needs a trained personnel. So, Anti-MCV antibody ELISA test is better for early diagnosis, follow up and assessment of therapy response. Further studies are needed for evaluation of different RA markers in order to reach early diagnosis and treatment for this disabling disease.*

INTRODUCTION

RA is a systemic autoimmune disease characterized by chronic joint inflammation that ultimately leads to joint destruction [1].Although the exact etiology of RA is still unknown, genetic predisposition, environmental factors like infectious agents or smoking and sex hormones may be all involved.Early definitive diagnosis is essential in RA patients,as they have a true chance for achieving a control of the disease if they are treated early and aggressively in the "window of opportunity" period. However, this needs sensitive clinical and laboratory diagnostic tools.[2,3]

RA has been associated with several autoantibodies, including RF, anti-perinuclear factor (APF), anti-keratin antibodies (AKA) and anti-filaggrin antibodies (AFA)[4]. AKA is an antibody that reacts with the keratinised tissue of rat oesophagus[5]. In the last few years anti-citrullinated peptide antibodies including antibodies to filaggrin, fibrin and vimentin resulting in

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the commercially available anti-cyclic citrullinated peptide antibody assay[6]. Citrullination is a posttranslational protein modification characterized by the conversion of positively charged arginine amino acid residues into neutrally charged citrulline. This process is performed by the calcium-dependent peptidyl arginine deiminase (pAD) enzyme family, with certain isotypes being expressed in monocytes (PAD4) and macrophages (PAD4 and PAD2)[7]. Citrullination is upregulated by inflammation and found to increase immunogenicity of proteins in collagen-induced arthritis mouse models [8]. Citrullinated antigens are thought to play a pivotal role in the pathogenesis of RA as they are expressed in inflamed joints and anti-citrullinated protein antibodies are present before the onset of clinical disease[9].

METHODOLOGY

This work was carried out in Microbiology and Immunology Department, Benha Faculty of Medicine in the period between May 2014 and May 2015. It included 30 RA patients attending the Outpatient Clinic of Rheumatology and Rehabilitation Department-Benha University Hospital; 28 females and 2 males, their age ranged from 22 to 60 years with mean of 43.27±13, They were fulfilling the 2010 American College of Rheumatology/European League Against Rheumatism classification criteria for rheumatoid arthritis (ACR/EULAR). Thirty patients; 28 females and 2 males, their age ranged from 17 to 50 years with mean of 35.93±9.74.They were diagnosed as other autoimmune rheumatic disease (20 patients: Systemic Lupus Erythematosus, 7 patients: Scleroderma and 3 patients Dermatomyositis) and 30 healthy control subjects;26 females and 4 males, their age ranged from 20 to 60 years matched for age and sex. Informed consent was obtained from patients and controls participated in this study. The age, sex, disease duration of the patients were recorded. Visual Analogue Scale of pain (VAS), disease activity score 28 (DAS28) and erythrocyte sedimentation rate (ESR) were used to assess disease activity. DAS 28 score is composed of four measuring parameters: 28 tender (TJC28) and swollen joint counts (SJC28), ESR, and patient global health assessment. Patients were grouped according to DAS28 scores as having high disease activity (DAS28 >5.1),moderate disease activity (3.2<DAS28<5.1) and mild disease activity (2.6<DAS28<3.2).

serum samples were collected from patient and control groups stored at -20°C until used for assay of anti-MCV and detection of AKA antibodies. Anti-MCV antibodies were measured using ELISA kits (ORGENTEC Diagnostica GmbH, Mainz, Germany), results are expressed in U/ml using a simple point-to-point curve-fitting method, values of 20.0 U/ml or greater were considered to be abnormal according to manufacturer's recommendations. AKA were detected by indirect immunofluorescent technique.

RESULTS

Table 1: Results of Anti-mutated citrullinated vimentin test (anti-MCV).

Study groups	***Anti-MCV (U/ml)***		χ^{2*}	***P-value***
	Range U/ml	**Mean ± SD**		
RA patients (no.=30)	5-1172	283.8± 320.88	48.19	<0.001 (HS)*
Other rheumatic disease patients (no.=30)	5-70	17.97± 14.02		
Control group (no.=30)	2-45	10.77± 8.28		

Table 1 shows that the mean serum level of Anti-MCV in RA patients was 283.8± 320.8 U/ml which was significantly higher (p<0.001) than in other studied groups.

This study showed that the sensitivity and specificity of anti-MCV test in RA patients at disease duration ≤2 years were 84.2% and 86% respectively. While at disease duration >2 years the sensitivity and specificity of anti-MCV test were 90 % and 78.3% respectively.

serum levels of anti-MCV correlated insignificantly with the age of RA patients (P > 0.05) and significantly correlated with each of the duration of the disease, DAS 28 ,VAS and ESR (p<0.05) as shown in table 2.

Table 2: Correlation coefficient (r) between demographic , clinical and laboratory data versus serum anti- MCV level in RA patients.

demographic, clinical and laboratory data	***Spearman's correlation coefficient (rho; ρ)***	***P-value***
Age (years) (no.=30)	(-) 0.28	0.13 (NS)
Duration of illness (years)	0.72	<0.001 (HS)
DAS	0.90	<0.001 (HS)
VAS	0.95	<0.001 (HS)
ESR	0.69	<0.001 (HS)

HS: highly significant.

Table 3: Results of anti-keratin antibodies (AKA) test:

AKA	***RA patients (no.=30)***		***Other rheumatic patients (no.=30)***		***Control group (no.=30)***		***Total (no.=90)***		χ^2	***P-value***
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Positive	22	73.33	9	30.00	10	33.33	41	45.56	14.07	<0.001*
Negative	8	26.67	21	70.00	20	66.67	49	54.44		(HS)

Table 3 shows that out of RA patients 22 (73.33%) were AKA positive and 8 (26.67%) AKA negative. Out of the other group of patients 9 (30%) were AKA positive and 21 (70%) AKA negative. In healthy control group 10 (33.33%) were AKA positive and 20 (66.67%) AKA negative. So, out of total 90 studied cases 41(45.56%) were AKA positive and 45 (54.44%) AKA negative. The comparison of AKA in RA and other groups show high statistical significant difference.

Our study showed that the sensitivity and specificity of AKA test in RA patients at disease duration ≤2 years were 68.4 % and 68.3 % respectively. While at disease duration >2 years the sensitivity and specificity of AKA test were 81.8 % and 68.3 % respectively.

There was insignificant correlation between each of sex, age, duration of illness, ESR and serum AKA and significant correlation between DAS, VAS and serum AKA as shown in table 4.

Table 4: Demographic, Clinical and Laboratory data versus serum AKA test results in RA patients:

Demographic, Clinical and Laboratory data		***AKA test results***		***Test***	***P-value***
		Positive (no.=22)	**Negative (no.=8)**		
Sex	Female (%)	20 (90.91)	8 (100.0)	FET	1.00
	Male (%)	2 (9.09)	0 (0.0)		
Age (years)	mean± SD; (range)	42.32±13.01; (22-60)	45.87±13.46; (28-60)	t= 0.66	0.52
Duration of illness (years)	mean± SD; (range)	2.32±1.13; (1-4)	1.44±1.12; (0.5-4)	t=1.89	0.07
ESR	mean± SD; (range)	42.54±10.82; (25-68)	36.5±9.35; (26-50)	t=1.4	0.17
DAS	mean± SD; (range)	3.94±0.96; (2-6.2)	2.81±0.71; (2-4.2)	t=3.02	0.005 (S)
VAS	mean± SD; (range)	6.05±2.31; (2-10)	3.29±1.85; (0-6)	t=3.04	0.005 (S)

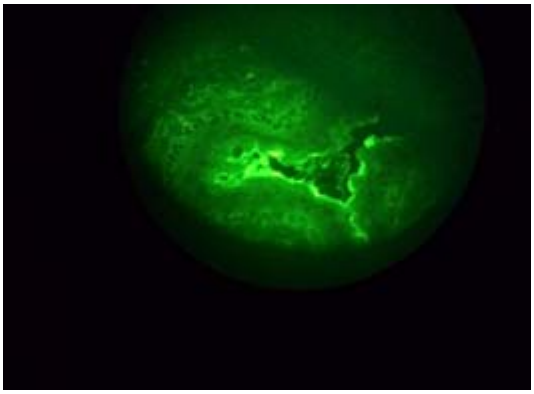

Fig. 1: Positive AKA IIF test.(400x)

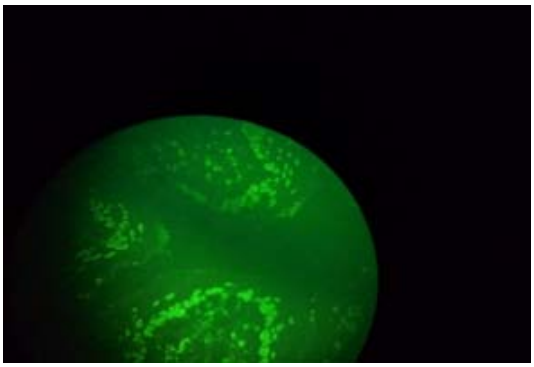

Fig. 2: Negative AKA IIF test.(400x)

DISCUSSION

Numerous serological markers of RA have been described, there are continuous efforts directed towards identifying the potentially pathogenic molecules and autoantibodies that are involved in the disease process and their contribution to disease patterns, severity, progression and prognosis with treatment with an additional focus on the role of the sensitivity and specificity of new disease specific autoantibodies in establishing early diagnosis that requires early therapeutic intervention. Therapeutic intervention early in the course of RA leads to more efficient disease control, less joint damage, and better prognosis of disease outcome.[10,11]

Several reports have demonstrated high diagnostic value of antibodies directed against citrullinated proteins in the diagnosis of RA[12].

Our study showed that the serum levels of anti-MCV were significantly increased in RA patients in comparison with other groups, which agrees with the reports of Bang et al[13], Hamdy et al [11] and Ismail et al[14]. These results support the hypothesis that citrullinated vimentin plays an integral role in triggering the inflammatory immune response in RA[15].

In contrast to this finding, Morbach et al[16] found insignificant statistical difference in anti-MCV serum levels in RA patients and other groups, which was explained by the fact that vimentin contains 43 arginine residues with 10 citrullination sites. Anti- MCV antibodies are considered a heterogenous group of antibodies directed against different epitopes on the citrulline molecule[3].

In the present study, anti-MCV titre was highly significantly correlated with ESR, DAS-28, VAS in RA patients. Such findings are consistent with those reported by Innala et al [17], Sirayildiz et al [18] and Zhu and Feng[19] who concluded that anti-MCV titres correlated significantly with DAS-28, VAS and ESR. Keskin et al [15] in a three year follow-up study of 427 RA patients found that patients with active RA had higher anti-MCV titers compared to patients with inactive disease.

Hamdy et al [11] reported that serum level of anti-MCV didn't show any significant variations with age, disease duration or ESR in RA patients, but it correlated significantly with DAS28 and VAS.

In our work the sensitivity and specificity of anti-MCV test in RA patients at disease duration ≤ 2 years were 84.2 % and 86 % respectively.While at disease duration 2 years the sensitivity and specificity of anti-MCV test were 90 % and 78.3 % respectively.

Dejaco et al [20] reported that anti-MCV sensitivity and specificity were 69.5 % and 90 % respectivly. Bang et al [3] reported that the sensitivity and specificity were 82% and 88% respectively. Hamdy et al [11] in their study reported that anti-MCV had diagnostic sensitivity and specificity of 75.5% and 93.3% respectively. Ismail et al[14] reported that the sensitivity and specificity of anti-MCV were 84% and 80% respectively. Lee and Bae [21] included in their study 2003 RA patients and 831 healthy controls, they reported that the sensitivity and specificity of anti-MCV were 86.6% and 94.2% respectively. Such variations in the sensitivity and specificity might be attributed to the fact that some of the studies included patients with undifferentiated arthritis and psoriatic arthritis [22].

Although the specificity of anti-MCV was more than 90% in most studies, the sensitivity of the same antibodies varied between 33% and 87.2%, possibly reflecting diverse genetic backgrounds and/ or methodological differences in diverse antigen preparations and detection techniques applied.[12]

In this study,AKA show high significant statistical difference between RA patients and other studied groups.($p < 0.001$),as 22 (73.33%) out of 30 RA patients were AKA positive and 9 (30%) out of 30 other rheumatic disease were AKA positive and 10 (33.33%) out of 30 control subjects were AKA positive. This agrees with Sharma et al [23].

Mohamed et al [24] reported that the frequency of AKA positive RA patients was 76.7% in comparison to 50 % of other rheumatic diseases and 30% of healthy controls.

In the present work, There was insignificant correlation between each of age, sex, duration of illness, ESR and serum AKA ($p>0.05$ %).There was a significant correlation between each of DAS, VAS and

serum AKA (p=.005). Scott et al[25] didn't find any correlation of AKA and each of ESR and disease activity. Aly et al [26] reported insignificant correlation of AKA and each of age, disease duration and significant statistical differences between AKA and ESR. Mohamed et al[24] reported insignificant correlation between each of age, sex, disease duration and serum AKA and high significant correlation of DAS, ESR and serum AKA ($P < 0.001$).

In our study the sensitivity and specificity of AKA test in RA patients of disease duration ≤ 2 years were 68.4 % and 68.3% respectively.While,in RA patients of disease duration > 2 years the sensitivity and specificity of AKA test were 81.3% and 68.3% respectively. Goldbach-Mansky et al [4] reported that AKA sensitivity and specificity were 26% and 84% respectively. Aly et al [26] reported that AKA sensitivity was 48.5% and specificity was 95.8%. Mohamed et al[24] reported that AKA specificity was 70 %. Zhu and Feng[19] reported AKA had a sensitivity of 48.2% and specificity 97.6%.

The variations in the results of different studies may be due to differences in technique and in interpretation of AKA reactions such as absence of any grading of staining intensity [25].

CONCLUSIONS

From our study, it can be concluded that our results support the hypothesis that anti-MCV and AKA have a role in the pathogenesis of RA. The serum level of anti-MCV antibodies was significantly higher in RA patients than in healthy controls. Anti-MCV and AKA could be a useful marker for early diagnosis and follow up of RA patients, they also reflect both activity and severity of RA.Anti-MCV antibody ELISA test was more sensitive and specific than AKA IIF test.It is a quantitative test while AKA IIF is a screening test and needs a trained personnel. So, Anti-MCV antibody ELISA test is better for early diagnosis, follow up and assessment of therapy response. Further studies are needed for evaluation of different RA markers in order to reach early diagnosis and treatment for this disabling disease.

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