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Research Article

SEROPREVALENCE OF ANTI-CARDIOLIPIN ANTIBODIES IN SUDANESE PATIENTS WITH RHEUMATOID ARTHRITIS

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ABSTRACT

Background: Antiphospholipid antibodies are a heterogeneous circulating immunoglobulins family of approximately twenty auto antibodies directed against phospholipids binding plasma proteins. Anticardiolipin (ACL) is the most commonly investigated APL in relation with several diseases as Rheumatoid arthritis, systemic thrombosis, cerebral ischemia, deep vein thrombosis. Anti-cardiolipin antibodies can be classified in two ways; As IgM, IgG or IgA and As ß2-glycoprotein dependent or independent. Rheumatoid arthritis is a systemic autoimmune disease that presents as a symmetrical inflammatory polyarthritis which affects the smaller joints such as hands and feet first, before affecting larger joints and there are six main types of arthritis.

Objective: This study aimed to investigate the Seroprevlance of ACL among Rheumatoid arthritis patients.

Materials and Methods: This is a case-control study conducted in an Integrated Mdedical Lab and Al-borg Medical Laboratories in Khartoum, sudan, from September to November 2016. It included ninety subjects; 60 of them were cases and 30 were control subjects, Physical examination, IgG and IgM anti-cardiolipin assay investigated using in vitro ELISA and WBCs, PLT, Hb, HCT and RBCs using Sysmex ® Kx21-N hematology analyzer were done for each subject.

Results: Mean age among control was (42±18) and (42±14) among cases.Hb, HCT RBCs, WBCs and PLT were within the normal range but among control were higher than cases with significant association except WBCs; p= (0.000), (0,000), (0.000), (0.056), (0.002) respectively. The majority IgG and IgM were seronegative with significant association p= (0.015) and (0.005) respectively. Finally, a significant correlation was appeared between age and Hb, WBCs and PLT at 0.05 level (r= 0.329*,p=0.010), (r= 0.270*, p= 0.037), (r= -0.258* p= 0.047) respectively and Hb with both WBCs and RBCs; (r= 0.286*, p=0.027), a weak negative relationship between TWBCs and PLT at 0.01 level (r= 0.403**, p= 0.001).

Conclusion: This study concluded that there was a strong effects of rheumatoid arthritis activity on both ACA and WBCs, PLT, Hb, HCT and RBCs normal ranges.

INTRODUCTION

Antiphospholipid antibodies are a heterogeneous circulating immunoglobulins family of approximately twenty auto antibodies directed against phospholipids binding plasma proteins. Anticardiolipin (ACL) is the most commonly investigated APL in relation with several diseasesas Rheumatoid arthritis, systemic thrombosis, cerebral ischemia, deep vein thrombosis, pulmonary embolism and myocardial infarction. The three most clinically significant are lupus anticoagulant, anticardiolipin antibodies and anti-B2 glycoprotein I antibodies (Jivraj, 2009; Terashi *et al*., 2005).

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Cardiolipin is a phospholipid found in inner mitochondrial membrane primarily, but it is also a minor constituent of mammalian membranes in general. Where it constitutes about 20% of the total lipid composition. It can also be found in the membranes of most bacteria,which has been founded in 1-5% of systematically healthy population. The name 'cardiolipin' is derived from the fact that it was first found in animal hearts. It was first isolated from beef heart in the early 1940s, and it is essential for the optimal function of numerous enzymes that are involved in mitochondrial energy metabolism (Pangborn, 1942; Faghihi *et al*., 2009). Anticardiolipin antibodies (ACL) are most frequently determined antiphospholipid antibodies (Nash *et al*., 2004). ACL found in patients with; rheumatoid arthritis, systemic lupus erythematosus, antiphospholipid syndrome (APS), and some infectious diseases (Tincani *et al*., 2010). Within normal population, the frequency of ACL ranges between 1% in normal pregnancies and 5.6%, in blood donors

(Al Jabri, 2004). Increased levels of ACL were found in acute infections (up to 32%), in rheumatoid arthritis (4% - 25%), in medication-induced lupus (47%) and also in elder people without any characteristic symptoms (51%) (Slavica *et al*., 2005). In most cases, their presence is accompanied with arterial and/or venous thrombosis and recurrent fetal loss (Koike, 2000; Locht, 2006). Anti-cardiolipin antibodies can be classified in two ways; As IgM, IgG or IgA and As ß2-glycoprotein dependent or independent. In autoimmune disease ACA are beta-2 glycoprotein dependent, syphilis ACA, are beta-2 glycoprotein independent and can be assayed using the Venereal Disease Research Laboratory test (Hasan, 2010). However, further investigations demonstrated that ACL were directed against cardiolipin antigen complexed with a plasma protein as a cofactor, known as b2-glycoprotein I (b2-GPI) (Chighizola, 2014).

The etiology of these antibodies is not known. Furthermore, questions remain regarding the critical titer and persistence of the antibodies, as well as the optimal type, intensity, and duration of secondary preventive therapy (Barbui, 1994). Elevated titers of anticardiolipin antibodies may be present before the first occurrence of venous thromboembolism (Ginsburg, 1992). The presence of antiphospholipid antibodies is associated with a higher risk of thrombotic recurrence, and prolonged oral anticoagulation is recommended (Asherson, 1991; Long, 1991; Rosove, 1992). The pathogenic role of ACL antibodies in the induction of thrombosis and fetal loss has been clearly demonstrated in experimental animal models The effects of aβ2Glyp1antibodies on endothelial cell activation, trophoblast cells and induction of fetal loss in experimental animal models also suggest a direct pathogenic role of these antibodies in the development of the antiphospholipid syndrome (APS). [Sherer, 2007] A high seroprevalence of APL was observed in inhabitants of regions such as tropical Africa, where there is high endemicity of infectious diseases (Adebajo, 1993; Arviex *et al*., 2002). Although these infections can induce APL expression, the increase in antibodies is not often accompanied by any manifestation of the antiphospholipid syndrome (APS) such as thrombosis (Asheron, 2003). Rheumatoid arthritis (RA) is a chronic destructive inflammatory disease characterized by the accumulation and persistence of an inflammatory infiltrate in the synovial membrane that leads to synovitis and the destruction of the joint architecture. Rheumatoid arthritis (RA) is the commonest inflammatory joint disease, affecting approximately 1% of adults in the developed world (Isaacs *et al*., 2011). There are six main types of arthritis. Rheumatoid arthritis is a systemic autoimmune disease that presents as a symmetrical inflammatory polyarthritis which affects the smaller joints such as hands and feet first, before affecting larger joints (Symmons *et al*., 2000). Osteoarthritis is characterized by degeneration of the joints such as the knee and the hip (Cameron *et al*., 2013). Juvenile arthritis comprises a range of arthritic disorders affecting children and adolescents below the age of 16 years (Petty *et al*., 1997). Psoriatic arthritis is a form of arthritis affecting people with psoriasis, a skin disorder (Moll, 1973). Gouty arthritis, or simply gout, is associated with the deposition of monosodium urate crystals in the tissues and joints (Wallace *et al*., 1977). Ankylosing spondylitis is an axial arthritis, which affects the vertebra causing inflammatory spinal pain and limited spinal and chest wall movements (Malinowski, 2015).

It is well known that autoimmune disorders are involved in the pathogenesis of rheumatoid arthritis (RA). Different types of autoantibodies can be detected in the serum of patients with RA (Olech, 2006; Filipowicz‐Sosnowska *et al*., 2007), (30).including immunoglobulin M rheumatoid factor (IgM‐RF) and anticitrullinated protein antibodies (ACPA), which are serological markers of RA and play an important role not only in the diagnosis but also in the assessment of disease activity and prognosis (Ylli, 2008; Nakamura *et al*., 2005). These antibodies may occur not only in connective tissue diseases, but also in other disorders, antiphospholipid syndrome, other autoimmune diseases, infections, neoplasms, and as a result of drug use (Abaci, 2010)The estimated prevalence of APL in RA patients varies from 4% to 49%, with the average prevalence of 28% and median prevalence of 22%, based on the data collected from several studies (Olech, 2006). A study included 27 cross-sectional studies (20 population-based and 7 hospital-based) from Africa reporting on the prevalence of arthritis. The majority of the studies were from South Africa (44.4%) (Usenbo *et al*., 2015). Rheumatoid arthritis (RA) occurs worldwide with prevalence of 1% in the population, most common in females (Symmons, 2002), affecting women three times more than men (Symmons *et al*., 1994). It is estimated that arthritis and other rheumatic conditions affect 42.7 million Americans (Treister Nanthaniel, 1999) with prevalence of 0.5 to 1% in Western population. (39)Sudanese patients with RA have significantly higher disease activity and are often IgM-RF seronegative. Together with reports from Uganda and Cameroon, our data indicate a cluster of highly active and often seronegative RA in central Africa (Elshafie, 2016). In this context we aimed to investigate, in a prospective hospital-based study, the prevalence of ACL in patients admitted with Rheumatoid arthritis and in other patients admitted during the same period of time with common infections. In addition we aimed to investigate the prevalence ofACL and Hb, HCT, PLT, RBCs and TWBCs andtheir correlation with rheumatoid arthritis.

MATERIALS AND METHODS

This is a prospective case-control hospital-based study of the prevalence of anticardiolipin antibodies in Sudanese patients with Rheumatoid arthritis. Sixty patients with Rheumatoid arthritis were taken as a cases. And a group of thirty (Filipowicz‐Sosnowska *et al*., 2007) healthy people as a control group. This study conducted in Integrated Mdedical Lab and Al-borg Medical Laboratories in Khartoum, Sudan from September to November 2016. Total coverage sampling was evaluated, this study included Patients (20-60 years old) diagnosed with RA and control group Absence of rheumatoid arthritis, smoking which might have affected the progression of Rh.

Hematological assay

WBCs, PLT, Hb, HCT and RBCs were done using hematology analyzer (Sysmex Kx21-N-Japan) and IgG and IgM anti-cardiolipin assay using in vitro ELISA (Mindary reader) kit obtained from orgentecdiagnostika GmbH,Carl-Zeiess-str49-5155129 mainz- Germany was designed for the accurate quantitative measurement of IgG and IgM class antibodies against Cardiolipin in Human serum and plasma. A 96-well

plate had been pre coated with Cardiolipin and β2-Glycoprotein complex antigens to bind cognate antibodies. Controls, standards or test samples were added to the wells and incubated. Following washing, a horseradish peroxidase (HRP) labeled anti-Human IgG and IgM conjugate were added to the wells, which binds to the immobilized Cardiolipin-specific antibodies. TMB was then catalyzed by the HRP to produce a blue color product that changed to yellow after adding an acidic stop solution. The density of yellow coloration was directly proportional to the amount of Cardiolipin IgG and IgM sample captured in plate.

Data analysis

All statistical analyses were performed by SPSS software version 20. Continuous variables were expressed as mean and standard deviation. In the analytical phase, "t" test was applied to compare averages. Comparison between groups was performed with t-test (parametric variables). Correlations were determined and p-value < 0.05 was considered significant.

RESULTS

In this case-control study there were a 30 control subjects and 60 cases of rheumatoid arthritis attended to Integrated Mdedical Lab and Al-borg Medical Laboratoriesin Khartoum, Sudan.

Table 1. Shows mean and standard deviation of age

Parameter	Group	N	Mean±SD
Age	Control	30	42±18
	Case	60	42±14

Table (1) revealed that the mean age among control group, (n=30) was (42±18) whereas the mean among case group, (n=60) was (42±14).

Table 2. Mean levels of Hb, HCT, RBCs and PLT comparison among case and control group

Parameters	Group	N	Mean±SD	P-value
Hb	Control	30	13.84±1.30	0.000
	Case	60	11.01±0.51	
HCT	Control	30	40.90±4.34	0.000
	Case	60	36.68±0.81	
RBCS	Control	30	4.77±0.41	0.000
	Case	60	4.47±0.19	
TWBCS	Control	30	6.24±1.46	0.056
	Case	60	5.72±1.06	
PLT	Control	30	243.53±59.56	0.002
	Case	60	287.82±65.51	

Table (2) shows the mean and the relation between case and control of Hb, HCT,RBCs and PLT, it was as the following;

Hb mean among control and case groups was within the normal level; (13.84±1.30), (11.01±0.51), respectively with a strong association (0.000) p-value, HCT mean was (40.90±4.34) among control and (36.68±0.81) among case group with a strong association (0.000). In same results, the mean of RBCs (4.77±0.41) among control group and a little decreased (4.47±0.19) among cases, with a strong association (0.000). In addition, WBCs mean was (6.24±1.46) among control group and decreased among case group (5.72±1.06), p-value = (0.056). Finally, PLT mean (243.53±59.56) among case group and (287.82±65.51) among control, with significant association (0.002).

Table 3. Frequency and percentage distribution of IgG and IgM among case and control group

IgG		IgG		Total	P-value
		Positive	Negative		
Control	Frequency	4	26	30	0.015
	Percentage %	13%	87%	100%	
Case	Frequency	23	37	60	
	Percentage %	38%	62%	100%	
Total	Frequency	27	63	90	
	Percentage %	30%	70%	100%	
IgM	IgM				0.005
Control	Frequency	1	29	30	
	Percentage %	3%	97%	100%	
Case	Frequency	17	43	60	
	Percentage %	28%	72%	100%	
Total	Frequency	18	72	90	
	Percentage %	20%	80%	100%	

Table (3) shows IgG and IgM frequency and percentage distribution among case and control group, there was a (4) positive (13%) and (26) negative (87%) IgG among control, n= 30, on the opposite, (23) positive IgG (38%) and (37) negative IgG (62%) among cases, n= 60. There was a significant association between cases and control when p-value (0.015). According to IgM status; (97%) was negative among control and (72%) negative among case group, significant association showed (0.005) p-value between case and control group. Table (4) shows the most important findings that revealed the relationship between study variables using p-value and correlation coefficient, in this table there were a weak positive relationship between age and both Hb and TWBCs at 0.05 level (r= 0.329*, p= 0.010), (r=0.270*, p= 0.037) respectively, and weak negative relationship with PLT (r= -0.258* p= 0.047). In similar findings between Hb and both RBCs and WBCs there was a weak positive relationship (r= 0.286*, p=0.027) with same values. On the other hand there was a weak negative relationship between TWBCs and PLT at 0.01 level (r= -0.403**, p= 0.001).

DISCUSSION

Various reports on the prevalence of ACL in patients with RA are available in the literature. There was different incidence which is probably due to different patient selection criteria, disease duration and activity, methods of treatment, and diagnostic techniques.Firstly, in this study the mean age of patient in case group was (42±18) years and (42±14) among control group there were nether cases above 60 years norcases below 18 years Table (1). Furthermore, we studied the values of Hb, TWBCs, RBCs, HCT and PLT and investigate the association between case and control groups; all these parameters were within the normal ranges but there were a strong differences within case and control group as the following;the mean of Hbamong control group was higher than cases and there was a strong association, which means we accept the alternative hypothesis which revealed that; there is a strong association between disease and Hb values. In similar findings; HCT and RBCs have a similar resultsamong control higher than cases, and with a strong association.On the other hand, the mean of PLT level has a similar findings it was within the normal range among all cases, but case group has a

higher values than control group, p-value(0,002) revealed significant association among case and control. Finally, according to the mean of TWBCs among control was higher than case group, but on the opposite of previous parameters there was no statistically significant difference between case and control groups Table (2).

used in the measurement and ethnic racial differences with respect to investigators. Most large studies have reported only small percentages of RA patients with positive ACL antibodies (Harris, 1985). Finally by comparing study variables with each other's there were A good positive correlation by comparing age and Hb, And poor correlation was showed by comparing

Table 4. Relationship between study variables with age and each other

Parameters		AGE	HB	HCT	RBCS	TWBCS	PLT
Age	R-value		0.329*	0.223	-0.049	0.270*	-0.258*
	P-value		0.010	0.087	0.709	0.037	0.047
Hb	R-value	0.329*		0.073	0.286*	0.286*	-0.054
	P-value	0.010		0.582	0.027	0.027	0.684
HCT	R-value	0.223	0.073		0.203	0.104	-0.160
	P-value	0.087	0.582		0.120	0.429	0.221
RBCS	R-value	-0.049	.286*	0.203		-0.070	-0.028
	P-value	0.709	0.027	0.120		0.593	0.831
TWBC	R-value	0.270*	.286*	0.104	-0.070		-0.403**
S	P-value	0.037	0.027	0.429	0.593		0.001
PLT	R-value	-0.258*	-0.054	-0.160	-0.028	-0.403**	
	P-value	0.047	0.684	0.221	0.831	0.001	

** Correlation is significant at the 0.01 level (1-tailed)
* Correlation is significant at the 0.05 level (1-tailed

Furthermore, we studied the association between the presence of anticardiolipin antibody among rheumatoid arthritis cases and control group. The literature, Usenbo *et al*. (2015) confirmed the lack of prevalence data on arthritis in Africa, and has inadvertently exposed the question of reliability of the available data. Available reports on arthritis are too old to reflect present trends of the disease. The African League of Associations for Rheumatology is encouraged to lead a solution to the need of a Standard Demographic Health Survey in the five regions of Africa, using standardized diagnostic criteria, where applicable, which would help to fill these gaps and address the true burden of arthritis in Africa (Usenbo *et al*., 2015). In the present study, the prevalence of ACL in RA Sudanese patients; IgM seronegative was showed the highest majority among case and control, on the other hand a fewer Sudanese patients with RAwere IgM seropositive among cases with significantly association which revealed the huge differences between case and control group. These findings consistent with Elshafie *et al*. (2016), that revealed; Sudanese patients with RA have significantly higher disease activity and are often IgM-RF seronegative.

Together with reports from Uganda and Cameroon, our data indicate a cluster of highly active and often seronegative RA in central Africa. (40)Significantly a fewer Sudanese patients with RA were IgM-RF–positive, agreeing with previous studies from other countries in west and central Africa: Nigeria (48% and 13% seropositive) (Adebajo, 1991), (Greenwood, 1969). Congo (33%) (34), Uganda (28%) (Kanyerezi *et al*., 1970), and Zimbabwe (37%) (Davis *et al*., 1989), but differing from western Africa: Senegal (84% in rural areas and 88% in urban areas),(Lekpa *et al*., 2012) and Morocco (78%) (Ibn Yacoub, 2012), or from eastern Africa: Kenya (77%) (Houba *et al*., 1979). In addition, IgG antibodies prevalence also was seronegative 86.7% among control group and 61.7% among RA cases with significant association p-value (0.015) which means there was an effect of RA on IgG status among cases. Different study, Fort *et al*. (1987). (48)in contrast with these findings which revealed surprising high frequency of ACL antibody positivity in patients with RA, the differences of the results may be due to the different populations, the modality

age and TWBCs, on the opposite there was a poor negative correlation between age and PLT. in addition, a poor positive correlation appeared between Hb and both RBCs and TWBCs significant at the 0.01 level. Also poor negative correlation was significant at 0.05 level between Hb and PLT. FinallyThere was no correlation between the rest of study parameters of case and control groups among Sudanese population.

Conclusion

The prevalence of ACL is relatively high in patients with RA. There is a relationship between ACL among control and case group of Rheumatoid arthritis patients; however, we confirmed different correlations between age and both Hb and TWBCs and PLT, Hb and both RBCs and TWBCs and PLT.

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