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Determination of Rheumatoid Factor in Suspected Rheumatoid Arthritis Patients Attending Selected Hospitals in Zaria, Nigeria

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Abstract
The study was carried out to provide information on RF among RA especially in the Northern part of Nigeria is relatively scarce. As such, rheumatoid factor levels in blood could serve as a predictive index for the onset of Rheumatoid arthritis. A total of 182 sera samples were analyse for the presence of IgM rheumatoid factor by ELISA. Seventeen (17) were found to be positive and 165 negative giving a prevalence of 9.3% and 90.7% respectively. The age range of 50 and above had the highest RF positive cases of 4.9%. This is followed by the eighteen (18) to thirty (30) age range. The association was not significant at p-value of 0.05. Similarly, the relationship between RF and gender showed that 5.5% (n=101) of females were positive while than 3.8% (n=81) of males were positive. The association was not significant. The highest participants had tertiary level of education which has a RF prevalence of 4.4%. Most of the participants that tested positive for RF were married (8.2%). There was no significant association between positivity for RF and level of education as well as marital status. There was however no statistical significant association between RF and the signs and symptoms (stiffness of joints, swelling around the joints, general fatigue, muscle pains, fever 13 and joint pains). Out of the 29 patients with history investigated for RA, 4 (2.2%) were positive for RF. Among the participants that smoke cigarette (n=4), none was RF positive. Both factors did not show significant association at a p-value of 0.05. The information in this study would serve as a predictive index for the onset of Rheumatoid arthritis.
Keywords: Rheumatoid Arthritis, Rheumatoid Factor, Auto-immune disease, synovial inflammation

1. Introduction:

Rheumatoid arthritis (RA) an auto-immune disease, is the most common form of polyarticular inflammatory arthritis characterized by persistent synovial inflammation, bony erosions and progressive articular destruction with varying degree of physical disability and pains (Alam *et al.,* 2011), induced by the production of auto-antibodies called rheumatoid factors (RF) that are reactive with determinants in the Fc region of IgG (Owen *et al.,* 2013). The classic rheumatoid factor is an IgM antibody that binds to normal circulating IgG, forming IgM-IgG complexes that are deposited in the joints, leading to the activation of the complement cascade by the classical pathway. It is this reaction that results in a type III hypersensitivity reaction and the attendant chronic inflammation of the joints (Owen *et al.,* 2013). Chronic rheumatoid arthritis sequelae is mediated by cells of the innate system predominantly the macrophages to be driven by macrophages in collaboration with T_{DTH} cells that are usually present in large numbers in rheumatoid synovium, and cytotoxic T cells. It is these cell interactions that contribute to chronic articular damage and the characteristic pains (Stewart, 2006).

RA is presumed principally by the on the presence of at least four parameters including morning stiffness for at least one hour, arthritis affecting three or more joints areas; namely: Proximal interphalangeal (PIP), Metacarpo phalangeal (MCP) and Metatarso phalangeal (MTP), wrist, elbow, knee or ankle observed by a physician, Arthritis of the Hand joints (wrist, MCP, PIP), Symmetric Arthritis, Rheumatoid nodules observed by a physician, Presence of RF, Radiographic changes (erosions and/or periarticular osteopenia) in the hand, wrist, PIP or MCP areas (van Venrooij *et al*., 2002).

The first immune abnormality described in patients with RA was the production of antibodies, so-called "rheumatoid factors," (RF), directed against the constant region of IgG (Weyand and Goronzy, 2006). Rheumatoid factors, a class of immunoglobulins (Igs) that have different isotypes and affinities, were first detected more than 70 years ago, but there is still much to discover about the mechanisms underlying their production, physiological role, and pathological effects (Dorner *et al.,* 2004). It is one of the seven classification criteria for RA proposed by ACR (Spiritus *et al*., 2004).

Patients with rheumatoid arthritis (RA) are at increased risk of developing infections and appear to be particularly susceptible to septic arthritis, osteomyelitis, and skin and soft tissue infections (Doran 2002), which are mostly caused by *Staphylococcus aureus*. This bacterium is responsible for up to 80% of joint infections in patients with RA (Goldenberg, 1998). It has been shown that *S. aureus* and other gram-positive bacteria are potent inducers of TNF-α secretion from macrophages (Keller *et al*., 1992), that TNF-α enhances killing of *S. aureus* by neutrophils (Ferrante *et al*., 1993), and that a local increase in TNF levels might improve host defenses against staphylococcal foreign body infections (Vaudaux *et al*., 1992). These observations suggest that inhibition of TNF-α affects the response of the body to colonization and infection with *S. aureus*. The study was carried out to provide information on RF among RA especially in the Northern part of Nigeria is relatively scarce. As

such, rheumatoid factor levels in blood could serve as a predictive index for the onset of Rheumatoid arthritis.

2. Material and Method

2.1 Study Area:

This study is carried out in Zaria, which is a major city in Kaduna State in Northern Nigeria. Formerly known as Zazzau, it was one of the original seven Hausa city-states. The 2006 Census population of Zaria was 408,198. It occupies an area of 300 km² at latitude: 11° 07' 51" N and longitude: 7° 43' 43" E. The study was conducted in two hospitals and a health service centre. These are Major Ibrahim Hospital, Sabon Gari; Ahmadu Bello University Teaching Hospital and Ahmadu Bello University Health Services, Sick Bay, Samaru –Zaria

2.2 Study Population

The study population comprised males and females of 18 years and above attending the selected hospitals with characteristic symptoms classical of rheumatoid arthritis based on the criteria approved by the American College of Rheumatology (ACR). A structured questionnaire was also administered on the patients to obtain the socio-demographic risk factors as well as clinical presentations of the patients. The criteria for inclusion are patients with symptoms of rheumatoid arthritis, inflammatory arthritis, degenerative arthritis, traumatic arthritis and all other join pains. All patients with symptoms other than the symptoms of rheumatoid arthritis, inflammatory arthritis, degenerative arthritis, traumatic arthritis, and all other join pains.

Ethical approval was sought for and granted from The Ahmadu Bello University Health Research and Ethics Committee, and Kaduna State Ministry of Health, Kaduna.

2.3 Sample Size

The sample size was determined using the following equation (Naing *et al.,* 2006):

$$n = \frac{Z^2 pq}{d^2}$$

Where n = sample size;
Z = standard normal distribution at 95% confidence limit=1.96;
p = prevalence or seroprevalence=12.3% (Adelowo *et al*., 2010);
q = 1-p;
d = absolute desired precision=0.05

Therefore; $n = \frac{(1.96)^2 x 0.123 x (1-0.123)}{(0.05)^2} = 165.75889344$; 166 samples

2.4 Collection of Sample

A total of 182 blood samples were collected from the Ahmadu Bello University Teaching Hospital, Ahmadu Bello University Health Service, and Major Ibrahim Hospital, Sabon Gari in Zaria. Four (4) ml of blood was collected from each patient. The sample was centrifuged to obtain the serum for Rheumatoid factor assay.

2.5 Determination of Rheumatoid factor using IgM ELISA

The sera were screened for IgM autoantibodies (Rheumatoid factor) using AccuDiag™ Rheumatoid Factor IgM (RF) ELISA Kit. The assay was carried out according to the manufacture's manual as follows: The test sera, Calibrator and Control sera were diluted with a dilution factor of 1:21 in the Serum Diluent Type II. To individual wells, 100 µL of the appropriate diluted Calibrator, Controls and patient sera were added. To the reagent blank well, 100 µL of the Serum Diluent Type II was added. The microtitre plate was incubated at room temperature (25ºC) for 30 minutes. Thereafter, the liquid from each well was shaken out. The wash buffer, 300µL was added to each well, the plate wash shaken out and turned upside and blotted on paper towelling to remove all liquid. The wash was repeated four (4) times. One hundred (100) µL of the conjugate was added to each well, including the reagent blank well. The plate was then incubated at room temperature for 30 minutes. The wash procedure as described above was repeated. Thereafter, 100 µL of chromogen/substrate solution (TMB) was added to each well, including the reagent blank well and the plate incubated at room temperature for 15 minutes. The reaction was stopped by the addition of 100 µL of Stop Solution (1N H_2SO_4) following the same order of Chromogen/Substrate addition including the reagent plank well. The plate was tapped gently along the sides to mix the contents of the well and the plate was read on ELISA plate reader at a wavelength of 450 nm. The result was calculated based on the manufacturer's manual; where the Mean Calibrator Optical Density (O.D.) and the Correction Factor was used to determine Cut-off Calibrator Value; the Cut-off Calibrator Value together with the Optical Density obtained for patient sera was used to determine the Index Value given by the formula:

$$Cuttoff\ Calibrator\ Value = Correction\ Factor\ X\ Mean\ O.D.\ for\ Calibrator$$

$$Index\ Value = \frac{\text{O. D. obtained for patient sera}}{\text{Cuttof Calibrator Value}}$$

An index value ≤0.90 is negative and ≥1.10 is positive.

3. Result

3.1 Determination of Rheumatoid factor in the Study Population

The result of the rheumatoid factor test using ELISA kit is presented in Figure 1. A total of 182 sera samples were analyse for the presence of IgM rheumatoid factor. Seventeen (17) were found to be positive and 165 negative giving a prevalence of 9.3% and 90.7% respectively.

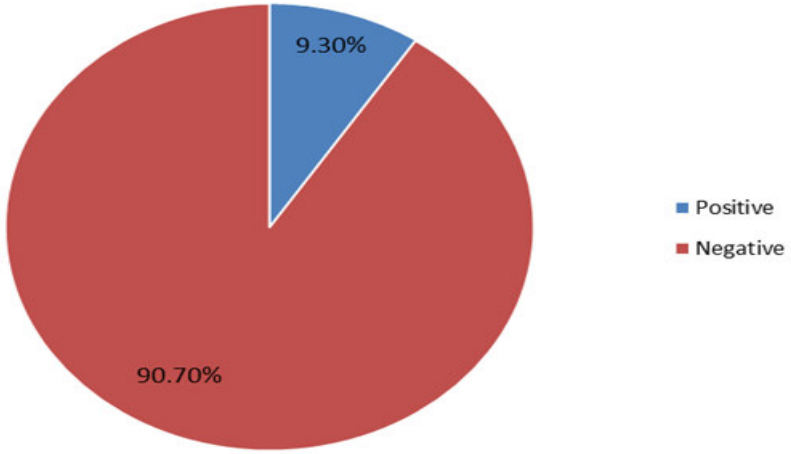

Figure 1: Prevalence of Rheumatoid factor in the Study Population

The relationship between the occurrence of Rheumatoid factor and demographic factors is presented in Table 1. The age range of 50 and above had the highest RF positive cases of 4.9%. This is followed by the eighteen (18) to thirty (30) age range; the least case, (0.0%), was observed in thirty six (36) to forty (40) age range. The association was not significant at p-value of 0.05. Similarly, the relationship between RF and gender showed that 5.5% (n=101) of females were positive while than 3.8% (n=81) of males were positive. The association was not significant. The highest participants had tertiary level of education which has a RF prevalence of 4.4%. Most of the participants that tested positive for RF were married (8.2%). There was no significant association between positivity for RF and level of education as well as marital status.

Table 1: The relationship between Rheumatoid factor and Demographic factors

Demographic factors	Frequency	Number of positive (%)	Number of negative (%)	Chi-square value	p-value*
Age					
18-30	30	2 (1.1)	28 (15.4)	6.588	0.361
31-35	18	1 (0.5)	17 (9.3)		
36-40	14	0 (0.0)	14 (7.7)		
41-45	26	1 (0.5)	25 (13.7)		
46-50	22	4 (2.2)	18 (9.9)		
>50	68	9 (4.9)	59 (32.4)		
No response	4	0 (0.0)	4 (2.2)		
Sex					
Male	81	7 (3.8)	74 (40.7)	0.084	0.772
Female	101	10 (5.5)	91 (50.0)		
Level of Education					
Primary	15	2 (1.1)	13 (7.1)	2.394	0.664
Secondary	20	2 (1.1)	18 (9.9)		
Tertiary	113	8 (4.4)	105 (57.7)		
Informal	33	5 (2.7)	28 (15.4)		
No response	1	0 (0.0)	1 (0.5)		
Marital status					
Married	147	15 (8.2)	132 (72.5)	0.713	0.700
Single	34	2 (1.1)	32 (17.6)		
Divorced	1	0 (0.0)	1 (0.5)		

* P-value significant at ≤ 0.05

The relationship between RF and signs/symptoms of RA is presented in Table 2. Participant with stiffness of joints record 11 (6.0%) positive cases, for, swelling around the joints 4(2.2%), general fatigue 16

(8.8%), muscle pains 15 (8.2%), fever 13 (7.1) and joint pains 14 (7.7) respectively. There was however no statistical significant association between RF and the signs and symptoms.The relationship between RF and factors predisposing to RA is presented in Table 3. Out of the 29 patients with history of being investigated for RA, 4 (2.2%) were positive for RF. Among the participants that smoke cigarette (n=4), none was RF positive as presented in table 4. Both factors did not show significant association at a p-value of 0.05

Table 2: The relationship between Rheumatoid factor and signs and symptoms of RA

Signs and Symptoms	Frequency	Number of positive (%)	Number of negative (%)	Chi-square value	p-value*
Stiffness of joints					
Yes	81	11 (6.0)	70 (38.5)	3.561	0.169
No	91	6 (3.3)	85 (46.7)		
No response	10	0 (0.0)	10 (5.5)		
Swelling around the joints					
Yes	41	4 (2.2)	37 (20.3)	0.976	0.614
No	137	13 (7.1)	119 (65.4)		
No response	9	0 (0.0)	9 (4.9)		
General Fatigue					
Yes	137	16 (8.8)	121 (66.5)	3.626	0.163
No	38	1 (0.5	37 (20.3)		
No response	7	0 (0.0)	7 (3.8)		
Muscle pain					
Yes	137	15 (8.2)	122 (67.0)	1.955	0.376
No	36	2 (1.1)	34 (18.7)		
No response	9	0 (0.0)	9 (4.9)		
Fever					
Yes	115	13 (7.1)	102 (56.0)	1.751	0.417
No	60	4 (2.2)	56 (30.8)		
No response	7	0 (0.0)	7 (3.8)		
Joint pains					
Yes	123	14 (7.7)	109 (59.9)	2.150	0.341
No	51	3 (1.6)	48 (26.4)		
No response	8	0 (0.0)	8 (4.4)		

Table 3: The relationship between Rheumatoid factor and factors predisposing to RA

Factors	Frequency	Number of positive (%)	Number of negative (%)	Chi-square value	p-value*
History of RA investigation					
Yes	29	4 (2.2)	25 (13.8)	1.417	0.492
No	145	13 (7.2)	132 (72.9)		
No response	7	0 (0.0)	7 (3.9)		
Smoking					
Yes	4	0 (0.0)	4 (2.2)	0.976	0.614
No	173	17 (9.3)	156 (85.7)		
No response	5	0 (0.0)	5 (2.7)		

* P-value significant at ≤ 0.05

4. Discussion

With respect to Rheumatoid arthritis, rheumatoid factor analysis of the patients involve in this work indicate an incidence of 9.3%. This low incidence could be as a result of seronegative RF situation among RA cases or the suspected individuals were non RA patients as suspected. Such an incidence agrees and disagrees with other studies. Adelowo and Bello, (2014) support this finding in their reported of a 38.5% prevalence of Rheumatoid factor in Nigeria. While Humphreys *et al*, (2014) reported a 34% in a study to determine the association of Rheumatoid factor and anti-citrullinated protein antibody positivity, but not level, with increased mortality in patients with rheumatoid arthritis. Our study is at variance to Silman *et al*, (1993) where they reported an absence of rheumatoid arthritis in a rural Nigerian population in their 2-stage population screening survey of 2,000 inhabitants of 2 rural townships in southern Nigeria.

The highest occurrence of RF was found among the age range of 50years and above. This could be as a result of immunity, which usually decreases with increase in age as. This is in agreement with van Schaardenburg *et al*, (1993) and Ceccato *et al*, (2006), who reported the increase in incidence of RF with

advance in age. Higher occurrence observed among women than men is also in concordance with Bushra (2008). Prevalence of RA is reported to be two to three times greater in women than men and although it may present at any age, it occurs mostly in the third to sixth decades of life (Ruffing and Bingham 2012). Concerning level of education, occurrence is high among the tertiary, this is probably because they comprise the highest number among the subjects in the study.

The signs and symptoms of RA were observed among the subjects. All of the signs and symptoms except swelling at the joints were found to be implicated here even though the association was not established. Among those previously suspected of RA (29), only 4 (2.2) are rheumatoid factor positive. However, a negative RF does not rule out RA; rather, the arthritis is called seronegative. This is the case in about 15% of patients (Nishimura *et al.,* 2007). During the first year of illness, rheumatoid factor is more likely to be negative with some individuals converting to seropositive status over time. Smoking as a factor to RA was not implicated in this study. However, other authors reported the contrary (Heliövaara *et al*., 2000, Mattey *et al*., 2002 and Klareskog *et al*., 2004). Also, a study found smoking to be a predictor primarily in the subset of patients with RA-associated HLA-DRB1 genotypes, indicating that genetic and environmental factors could interact in predisposing to RA (Gorman, 2006). Uncontrolled active rheumatoid arthritis causes joint damage, disability, decreased quality of life, and cardiovascular and other co-morbidities. Patients with (RA) are particularly susceptible to septic arthritis. The case fatality rate for bacterial arthritis has not changed substantially in the past 25 years. There has been misdiagnosis of RA with consequent wrong therapeutics.

Information on RF among RA especially in the Northern part of Nigeria is relatively scarce. As such, rheumatoid factor levels in blood could serve as a predictive index for the onset of Rheumatoid arthritis (Nielsen *et al.,* 2012). The appearance of an acute exacerbation in one or more joints in a patient positive for rheumatoid arthritis should arouse suspicion of infection. Any early onset or on-going infection previously known may be detected for effective therapy to avoid irreversible joint damage

5. Conclusion

The study establishes the presence of RF among the study population with a prevalence of 17%. It also confirms that presence of RF increases with age of the individuals. Most of those that tested positive for RF had tertiary education. Generally, the study did not establish any significant statistical association between RF positivity and the demographic and risk factors studied.

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