International Journal of Research in Medical Sciences
Yeldu MH et al. Int J Res Med Sci. 2017 May;5(5):1948-1952
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20171823

Original Research Article

Oxidative stress factors in Nigerians with rheumatoid arthritis

Yeldu M. H.[1]*, Arinola O. G.[2]

[1]Department of Chemical Pathology, Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto-Nigeria
[2]Department of Chemical Pathology and Immunology, University College Hospital, Ibadan- Nigeria

Received: 01 March 2017
Accepted: 31 March 2017

***Correspondence:**
Dr. Yeldu M. H.
E-mail: mhyeldu@gmail.com

ABSTRACT

Background: Rheumatoid arthritis (RA) is a chronic progressive inflammatory autoimmune disorder characterized by symmetric erosive synovitis and sometimes with multi-system involvement. But the exact mechanism of the disease is not fully understood. In the light of above explanation, the present study measured the plasma levels of total peroxide potential (TPP), total antioxidant potential (TAP), malondialdehyde (MDA), oxidative stress index (OSI) and nitric oxide (NO) in relation to the titer of rheumatoid factor among RA patients compared with controls.
Methods: This study included 28 rheumatoid arthritis patients and 28 apparently healthy subjects as controls who were matched for age (50-60 years), sex, and socioeconomic status. Rheumatoid factor was estimated using latex method as described by manufacturer. Anthropometric parameters and plasma levels of TPP, TAP, OSI, MDA and NO were determined using standard techniques.
Results: The result indicated that with the exception of mean body weight which was significantly ($p<0.001$) higher among RA patients (90.61±2.02 years) as compared with controls (77.91±2.51 years), mean age, height and body mass index of RA patients (55.68±1.05kg, 1.65±0.01m and 33.40±0.83 kg/m2 respectively) were not significantly different compared with controls (54.07±1.04kg, 1.61±0.02m and 30.44±1.28 kg/m2 respectively). Plasma TPP, NO, OSI and MDA were significantly ($p<0.01$; $p<0.001$) higher while, plasma TAP is significantly lower among RA patients compared with controls. Plasma MDA was positively correlated with titer of rheumatoid factor in the RA patients.
Conclusions: Our findings therefore may raise the concept that there are some yet unknown key events in the pathogenesis of RA determination of sex of the skull along with other parameters.

Keywords: Oxidative stress factors, Nigeria, Rheumatoid arthritis

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic progressive inflammatory autoimmune disorder characterized by symmetric erosive synovitis and sometimes with multi-system involvement.[1] But the exact mechanism of the disease is not fully understood. More so, rheumatoid arthritis has no complete cure.[2] Previous studies have shown that reactive oxygen species (ROS) play a key role in the pathogenesis of many diseases including RA.[3, 4] ROS production is normally controlled by a number of the body antioxidant defence system. These comprised of the enzymatic antioxidant which includes glutathione peroxidase (GPx), catalase, superoxide dismutase (SOD), glautathione-S-transferase (GST) and glutathione reductase (GR), while non- enzymatic antioxidant defence which includes retinol (vitamin A), ascorbic acid (vitamin C), α-tocopherol (vitamin E) reduced glutathione (GSH).[5] Imbalance between the antioxidants and oxidants which may occur from the excessive production of the oxidants or insufficient antioxidant defence system may result to oxidative stress.[4] ROS if not

adequately scavenged can damage a number of biomolecules including, DNA, proteins and lipids in joint tissues.[2, 6]

An epidemiological study suggested that low selenium status may be a risk factor for rheumatoid factor-negative RA and low alpha-tocopherol status may be a risk factor for RA independently of rheumatoid factor status.[7] Another study suggested that ROS generation can be decreased via inhibition of an enzyme (thioredoxin reductase) by gold thioglucose in RA.[8] Overall there are evidences that there is increased state of oxidative stress in RA, which proposes the use of antioxidant supplementation in such patients. Plasma catalase had been reported to be significantly lower in patients with RA.[9] Another study reported impaired glutathione reductase activity in synovial fluid in rheumatoid arthritis.[10] In active RA and juvenile idiopathic arthritis11 increased oxidative stress and decreased levels of antioxidants have been reported.

Although etiopathogenesis of RA is yet to be fully revealed, nonetheless literatures revealed that reactive oxygen species (ROS) play important roles in RA pathogenesis.[1,8-12] ROS, which can be produced as a result of normal aerobic metabolism and whose production is increased by active neutrophils during inflammation, have attracted increasing attention.[13] Oxidative stress generated within an inflammatory joint can produce autoimmune phenomena and connective tissue destruction in rheumatoid synovitis.[14] It is known that neutrophils in the joints are activated by infection and inflammation. Activated neutrophils lead to the production of ROS like hydrogen peroxide, hydroxyl radical and superoxide radical as well as, nitrogen containing radicals which in turn causes tissue injury.[1,2] Thus it may be hypothesized that oxidative stress is involved in the pathogenesis of RA. In the light of above explanation, the present study measured the plasma levels of TPP, TAP, OSI, MDA and NO in relation to the titer of rheumatoid factor in RA patients and compared with controls.

METHODS

The current study was conducted among rheumatoid arthritis patients who attended the Rheumatology Clinic of a tertiary Hospital at Ibadan, Nigeria. An institutional ethical clearance and informed consent was obtained from each subject prior to the commencement of the study. The study included 28 rheumatoid arthritis patients and 28 apparently healthy subjects as controls who were matched for age (50-60 years), sex, and socioeconomic status.

Diagnosis of RA patients was done on the basis of clinical history such as morning stiffness in and around joints, serological investigation like serum rheumatoid factor and radiographic changes viz. erosions or unequivocal bony decalcification on posterior-anterior hands and wrists.[15] Those having clinical history of the disease such as diabetes mellitus, cardiovascular diseases, hypertension, inflammatory diseases and infectious diseases were strictly excluded. Also excluded are cigarette smokers, alcoholics, and those compulsory medication, vitamin and mineral supplementation.

Anthropometric parameters were estimated using standard techniques. Subjects were weighted with minimum clothing to the nearest 0.1 kg by using a regularly calibrated weighing health scale; model ZT 120 (manufactured by Seca Gmbh and Co., Germany), while the heights were measured by using a calibrated Stadiometer, model 220 (manufactured by Seca Gmbh and Co., Germany). Body mass index (BMI) for each subject was calculated using the following formula: BMI (kg/m2)=Body Weight (kg)/Height (m2).

Blood samples were gotten from the Department of Chemical Pathology and Immunology, University College Hospital, Ibadan, Nigeria. About 5 ml of venous blood samples were collected in bottles containing lithium heparin and plasma were processed for rheumatoid factor using latex method as described by manufacturer. The plasma levels of total peroxide potential (TPP), malondialdehyde (MDA), total antioxidant potential (TAP), and the level of NO was estimated using Griess reagents method.[16-19] The ratio of the plasma TPP level to the TAP level was regarded as the oxidative stress index (OSI).

Statistics analysis

All results were expressed as means ±SD. Student's t-test and Spearman's correlation analysis were performed using SPSS for Windows Release 11.0 (SPSS Inc. Chicago, Illinois, USA). The P value less than 0.05 was considered to be significant.

RESULTS

The anthropometric parameters among rheumatoid arthritis patients and controls are presented in Figure 1.

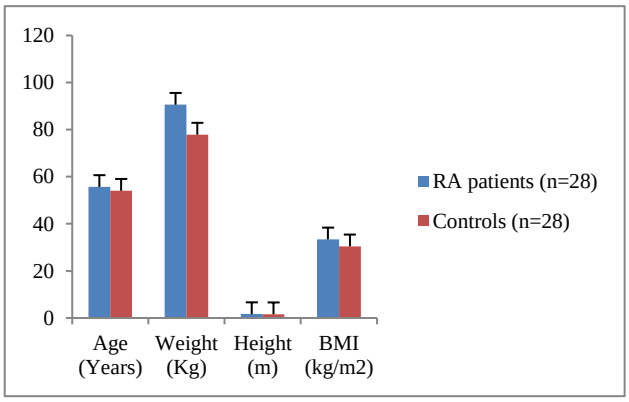

Figure 1: Anthropometric parameters among rheumatoid arthritis patients and controls.

The result indicated that with the exception of mean body weight which was significantly higher (90.61±2.02 years) in RA patients as compared with controls (77.91±2.51 years), the mean age, height and body mass index of RA patients (55.68±1.05kg, 1.65±0.01m and 33.40±0.83 kg/m2 respectively) were not significantly different compared with the corresponding control values (54.07±1.04kg, 1.61±0.02m and 30.44±1.28 kg/m2 respectively). The result of oxidative stress factors in rheumatoid arthritis patients and controls is presented in Figure 2.

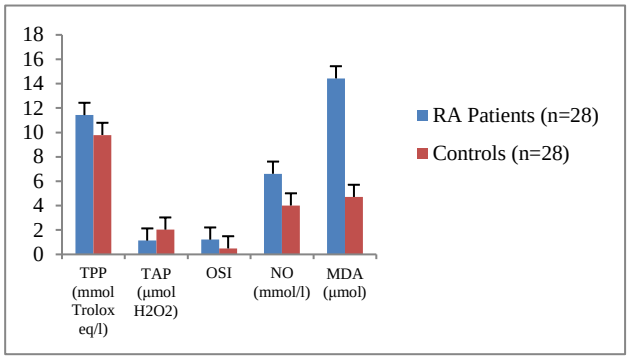

Figure 2: Oxidative stress factors among rheumatoid arthritis patients and controls.

The result indicated significantly ($p<0.01$; $p<0.001$) higher plasma TPP (11.43±0.42 mmol Trolox eq/l), OSI (1.21±0.08), NO (6.61±0.17mmol/l) and MDA (14.43±0.43µmol), while plasma TAP (1.13±0.05µmol H2O2) is significantly ($p<0.01$) lower among RA patients compared with normal healthy controls (9.79±0.3 mmol Trolox eq/l, 2.03±0.09 µmol H2O2, 0.49±0.04, 4.01±0.12 mmol/l and 4.71±0.21 µmol respectively). Table 1 shows the correlation between oxidative stress biomarkers and rheumatoid factors (RF). The result indicated that plasma MDA was positively correlated with titer of rheumatoid factor in the RA patients. The correlation of TAP with TPP, OSI, NO and MDA among rheumatoid arthritis patients is presented in Table 2 and Figures 3 to 4. The result indicated a significant ($r = -610$; $p<0.001$) negative correlation between plasma TAP and OSI. Significant ($r= -0.610$; $p<0.01$) negative correlation was also established between plasma TAP and TPP.

Table 1: Correlation between oxidative stress and Rheumatoid Factors (RF).

Parameter	Titre of RF
	r-value
TPP (mmol Trolox eq/l)	0.11
TAP (µmol H_2O_2)	0.21
OSI	0.24
NO (mmol/l)	0.22
MDA (µmol)	0.76*

Values are correlation coefficient (r); TPP=total peroxide potential; TAP= total antioxidant potential; OSI=oxidative stress index; NO=nitric oxide; MDA=malondialdehyde; *Significant positive correlation at the 0.01 level.

Table 2: Correlation of total antioxidant potential (Tap) with TPP, OSI, NO, MDA among rheumatoid arthritis patients.

Parameter	Correlation coefficient
	r-value
TPP (mmol Trolox eq/l)	r = - 0.53*
OSI	r = - 610**
NO (mmol/l)	r = 0.344
MDA (µmol)	r = 0.185

Values are correlation coefficient (r); TPP=total peroxide potential; OSI=oxidative stress index; NO=nitric oxide; MDA=malondialdehyde;*Significant negative correlation at the 0.01 level, ** Significant negative correlation at the 0.001 level.

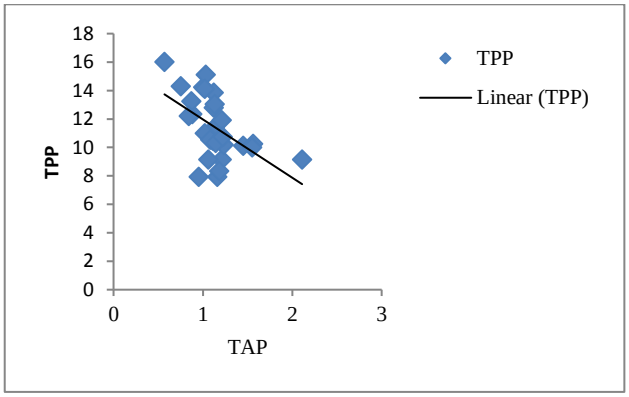

Figure 3: scatter plot of TPP and tap among rheumatoid arthritis patients.

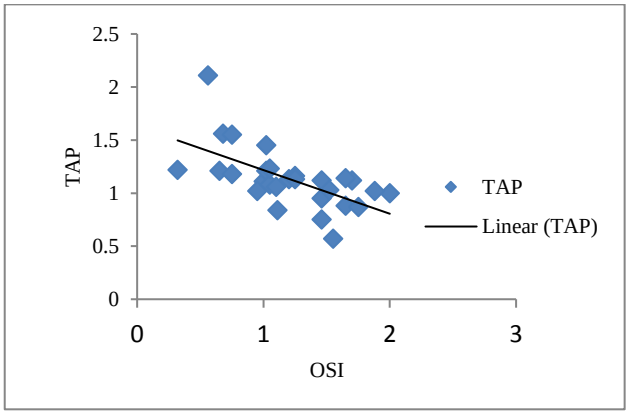

Figure 4: scatter plot of tap and OSI among rheumatoid arthritis patients.

DISCUSSION

Oxidative stress resulting from the excessive production of ROS is a deleterious process and may play a significant role in the pathogenesis of autoimmune disorders either by the breakdown of immunological tolerance, induction of apoptotic cell death and promotion of tissue inflammation.[20] In the current study, the oxidative stress biomarkers in plasma of patients that tested positive for rheumatoid factor and controls were evaluated. The results indicated enhanced oxidative stress

in RA patients as evidenced by increased total plasma peroxide, oxidative stress index, nitric oxide and decrease in plasma total antioxidant potential compared with controls.

Polyunsaturated fatty acids present in membrane lipids are the main target of attack by ROS leading to the formation of lipid hydrogen peroxide (LPO) and subsequently generating malondialdehyde (MDA).[9] It is known that activated inflammatory cells lead to ROS production.[1,13] and increased ROS, in turn, increase lipid peroxidation products and cause tissue injury.[1,9] Hydrogen peroxide and other derivatives of peroxides, produced physiologically diffuse into plasma. It is known that H2O2 and lipid peroxides increase in RA, therefore raised TPP in our RA patients might be the inevitable increase in lipid peroxides and ROS including H2O2 in RA.[2,21] Elevated levels of superoxide radical are produced by both the synovial fluids of macrophages and neutrophils of rheumatic patients provably because of their exposure to cytokines within the synovial fluids.[22] We observed significantly higher levels of NO in RA patients compared to controls. Reticular cartilage and synovial fibroblasts synthesize substantial amounts of nitric oxide and that the cause of inflammation in RA is a result of cytotoxic and cytostatic effects of nitric oxide.10 The increased plasma NO observed in RA patients might also be due to enhanced activity of the nitric oxide synthase, an enzyme responsible for the formation of NO.[23] Other studies have also reported similar findings.[24, 25] Therefore increased level of NO expected as shown by the present study.

Depolymerization and peroxidation of hyaluronic acid in synovial fluid result into loss of lubricating property of the fluid. It has been reported that, it is very important consequence of exposure of synovial fluid to superoxide and hydrogen peroxide.[26] MDA, the product of lipid peroxidation reacts with lysine residues in proteins to produce immunogenic molecules, which can exacerbate inflammation.[27] Thus, raised MDA in RA might have a direct link with pathogenesis of RA. Higher serum and synovial fluid concentrations of MDA have reported among patients with RA.[28] In the current study, plasma MDA concentration was significantly higher in RA patients compared with controls. Our results are in agreement with the previous studies, where elevated plasma MDA levels were reported among RA patients compared to controls. The increased plasma MDA concentration in RA patients could be due to excessive generation of ROS which progresses in chronic inflammation causing tissue damage.[2,5,25,29-32] The current study demonstrated that plasma MDA was positively correlated with titer of rheumatoid factor in the RA patients, while plasma TAP was negatively correlated with OSI and TPP. The established negative correlations observed in the current study suggest that RA patients are prone oxidative stress which leads to decreased total antioxidant capacity and increased lipid peroxidation products (MDA). The resulting oxidant/antioxidant imbalance could deleterious leading to tissue damage in RA patients. Thus, our results are in keeping with possible evidence of increased reactive oxygen/nitrogen species generation and tissue damage in RA. Increased generation of ROS lead to enhanced lipid peroxidation accompanied by elevated MDA concentrations in plasma of RA patients. It can therefore be deduced that, lipid peroxidation may play a significant role in the pathogenesis of RA.

CONCLUSION

The study raise the concept that oxidative stress as evidenced by increased MDA, NO, TPP, OSI and decreased TAP may play a key role in the pathogenesis of RA. The study also indicated a strong relationship between oxidative stress and rheumatoid arthritis and underscores the role of antioxidants in reducing oxidative stress associated with rheumatoid arthritis.

ACKNOWLEDGEMENTS

Authors wish to acknowledge with thanks the following individuals/intuitions whose contributions are immeasurable towards the success of this research work: (i) volunteers and research assistants that are too numerous to list here. (ii) Colleagues at the Faculty of Medical Laboratory Sciences, Usmanu Danfodiyo University, Sokoto (iii) Department of Chemical Pathology and Immunology, University College Hospital, Ibadan, and (iv) Research Ethics Committee for approving the protocol for the research work.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

REFERENCES

1. Fox DA. Etiology and pathogenesis of rheumatoid arthritis. In: Koopman WJ, Moreland LW, eds. Arthritis and allied conditions a textbook of rheumatology. Fifteenth ed. Philadelphia: Lippincot Williams and Wilkins; 2005:1089-1115.
2. Jaswal S, Mehta HC, Sood AK, Kaur J. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. Clin Chim Acta 2003; 338: 123-29.
3. Biemond P, Swaak AJ, Koster JF. Protective factors against oxygen free radicals and hydrogen peroxide in rheumatoid arthritis synovial fluid. Arthritis Rheum 1984; 27:760-65.
4. Ozturk HS, Cimen MYB, Cimen OB, Kacmaz M, Drek J. Oxidant/antioxidant status of plasma samples from patients with rheumatoid arthritis. Rheumatol Int 1999;19:35-7.
5. Mateen S, Moin S, Khan AQ, Zafar A, Fatima N. Increased Reactive Oxygen Species Formation and Oxidative Stress in Rheumatoid Arthritis. PLoS ONE 2016;11(4):e0152925.

6. Mahajan A, Tandon VR. Antioxidants and rheumatoid arthritis. J Indian Rheumatol Assoc 2004;12:139-42.
7. Knekt P, Heliovaara M, Aho K, Alfthan G, Marniemi J, Aromaa A. Serum selenium, serum alpha-tocopherol and the risk of rheumatoid arthritis. Epidemiology. 2002;11:402-05.
8. Kerimova AA, Atalay M, Yusifov EY, Kuprin SP, Kerimov TM. Antioxidant enzymes: possible mechanism of gold compound treatment in rheumatoid arthritis. Pathophysiology. 2002;7:209-13.
9. Kamanli A, Naziroglu M, Aydile K, Hacievliyagil C. Plasma lipid peroxidation and antioxidant levels in patients with rheumatoid arthritis. Cell Biochem Funct 2004; 22:53-7
10. Bazzichi L, Ciompi ML, Betti L, Rossi D, Melchiorre M, Fiorini G, et al. Impaired glutathione reductase activity and level of collogenase and elastase in synovial fluid in rheumatoid arthritis. Clin Exp Rheumatol 2002;20:761-66.
11. De Leo ME, Tranghese A, Passantino M, Modente A, Lizzio MM, Galeotti T, et al. Manganese superoxide dismutase, glutathione peroxidase, and total radical trapping antioxidation capacity in active rheumatoid arthritis. J Rheumatol 2002;29:2245-46.
12. Sattar N, McCarey DW, Capell H, McInnes IB. Explaining how "high-grade" systemic inflammation accelerates vascular risk in rheumatoid arthritis. Circulation 2003;108:2957-63.
13. Nurcombe HL, Bucknall RC, Edwards SW. Activation of the neutrophil myeloperoxidase-H2O2 system by synovial fluid isolated from patients with rheumatoid arthritis. Ann Rheum Dis 1991;50:237-42.
14. Vasanthi P, Nalini G, Rajasekhar G. Status of oxidative stress in rheumatoid arthritis. Int J Rheum Dis 2009;12(1):29-33.
15. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, et al. The American Rheumatoid Association 1987 revised criteria for classification of Rheumatoid Arthritis. Arthritis Rheum 1988;31(3):315-24.
16. Satoh K. Serum lipid peroxide in cerebrovascular disorder determined by new colorimetric method. Clin. Chim. Acta 1978;90:37-43.
17. Harma M, Harma M, Erel O. Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly 2003;133:563-66.
18. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277-85.
19. Laudanska E, Gwuzdz A, Brudel G, Rajchet J. Evaluation of Griess reagent and TTC tests in obsterics. Ginekol Pol 1970;41(8):857-62.
20. Valko M, Leibfritz D, Moncol J, Cronin MTD, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. Int J Biochem Cell Biol 2007;39:44-84.
21. Jasin HE. Mechanisms of tissue damage in rheumatoid arthritis. In: Koopman WJ, Moreland LW, eds. Arthritis and allied conditions a textbook of rheumatology. Fifteenth ed. Philadelphia: Lippincot Williams and Wilkins; 2005:1141-64.
22. Hitchon CA, El- Gabalawy HS. Oxidation in rheumatoid arthritis. Arthritis Res Ther 2004;6(6): 265-78.
23. Ueki Y, Miyake S, Dominica Y, Eguchi K. Increased nitric oxide levels in patients with rheumatoid arthritis. J Rheumatol 1996;23:230-36.
24. Mäki-Petäjä MK, Cheriyan J, Booth AD, Hall FC, Brown J, Wallace SML, et al. Inducible nitric oxide synthase activity is increased in patients with rheumatoid arthritis and contributes to endothelial dysfunction. Inter J Card 2008;129:399-405.
25. Ali AM, Habeeb RA, El-Azizi NO, Khattab DA, Abo-Shady RA, Elkabarity RH. Higher nitric oxide levels are associated with disease activity in Egyptian rheumatoid arthritis patients. Revista brasileira de reumatologia. 2014;54(6):446-51
26. Blake DR, Hall ND, Tredy DA, Halliwell B, Gutteridge JMC. Protection against Superoxide and hydrogen peroxide in synovial fluid from rheumatoid patients. Clin Sci 1981;61:483-86.
27. Darlington LG, Stone TW. Antioxidants and fatty acids in the amelioration of rheumatoid arthritis and related disorders. Bri J Nutr 2001;85:251-69.
28. Lunec J, Halloran SP, White AG, Dormandy TL. Free radical oxidation (peroxidation) products in serum and synovial fluid in rheumatoid arthritis. J Rheumatol 1981;8:233-45.
29. Özgüneş H, Gürer H, Tuncer S. Correlation between plasma malondialdehyde and ceruloplasmin activity values in rheumatoid arthritis. Clinical biochemistry. 1995;28(2):193-4.
30. Akyol O Işçi N, Temel I, Ozgoçmen S, Uz E, Murat M, Buyukberber S. The relationships between plasma and erythrocyte antioxidant enzymes and lipid peroxidation in patients with rheumatoid arthritis. Joint Bone Spine. 2001;68(4):311-7.
31. Sarban S, Kocyigit A, Yazar M, Isikan UE. Plasma total antioxidant capacity, lipid peroxidation, and erythrocyte antioxidant enzyme activities in patients with rheumatoid arthritis and osteoarthritis. Clin Biochem 2005;38(11):981-86.
32. El-barbary AM, Khalek MAA, Elsalawy AM, Hazaa SM. Assessment of lipid peroxidation and antioxidant status in rheumatoid arthritis and osteoarthritis patients. The Egyptian Rheumatologist 2011;33:179-85.

Cite this article as: Yeldu MH, Arinola OG. Oxidative stress factors in Nigerians with rheumatoid arthritis. Int J Res Med Sci 2017;5:1948-52.