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Total anti-oxidant status and C-reactive protein values in Nigerians with symptomatic primary osteoarthritis of the knee joint – an initial report

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ABSTRACT: Osteoarthritis is a degenerative as well as an inflammatory disorder of joints. The joint inflammation in this disease (as with inflammation in other organs of the body) leads to the production of acute phase proteins like C-reactive protein (CRP) along with the release of cytokines and neutrophils. The lysosomes released from the activated neutrophils result in the production of large amounts of free radicals which are harmful to the human body. Antioxidants on the other hand are substances that when present even in low concentrations avidly react with and annihilate free radicals before oxidative damage is inflicted on vital components of the cells. This case-control study was aimed at investigating the influence of age; body mass index, total antioxidant status (an indirect measure of total free radicals) and c-reactive protein (an acute phase reactant) on symptomatic osteoarthritis of the knee. Thirty five subjects with clinical and radiological features of osteoarthritis of the knee were recruited from the Orthopaedic clinic of the University College Hospital, Ibadan. Twenty healthy age-matched controls were recruited from the Surgical Outpatient Clinic of the same hospital. Informed consent, biodata and body mass indices were obtained after which venous blood samples were obtained from each subject. Total plasma antioxidant status (TAS) was estimated spectrophotometrically by the Koracevic method while the CRP concentration was measured using Humatex CRP kit® (Human GmbH, Germany). The mean ages of the test and control groups were 58.8±12.0 and 52.3±7.9 years respectively. Osteoarthritic patients demonstrated significantly higher (p<0.05) BMI (32.2±6.9 kg/m2) compared to controls (26.1±3.4 kg/m2). The mean TAS was significantly lower (p <0.05) in the test (1.09±0.38 mmol/l) compared to the controls (1.61±0.09 mmol/l). CRP was positive in 19 (54.3%) of the test subjects but negative in all the control. There was an inverse correlation between the BMI and TAS (p <0.05) as well as between age and TAS (p <0.05). This study shows that Nigerian patients with symptomatic osteoarthritis of the knee tend to be obese and have a lower total antioxidant status. Antioxidant supplementation of the diet as a means of minimizing the severity of osteoarthritis and other free radical-mediated diseases in older Nigerian adults is advocated.

Keywords: *Osteoarthritis; anti-oxidants; c-reactive protein; acute phase reactants; Nigeria.*

INTRODUCTION

At the workshop 'New horizon in osteoarthritis' jointly sponsored by the American Academy of Orthopaedic Surgeons, the National Institute of Arthritis, Musculoskeletal and Skin Diseases, the National Institute on Aging, the Arthritis Foundation and the Orthopaedic Research and Education Foundation held in 1994 in the United States of America, the consensus definition of osteoarthritis is that of 'a group of overlapping distinct diseases which may have different aetiologies, but with similar biologic, morphologic and clinical outcomes (Keuttner and Goldberg 1995). The disease processes in osteoarthritis does not only affect the articular cartilage, but involve all the structures in the entire joint including the subchondral bone, ligaments, capsules, synovial membrane and peri-articular

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muscles. However, taking into cognizance the initiation of the events leading to the observed joint changes in osteoarthritis, Kuettner and Goldberg defined osteoarthritis as a multifactorial disease of diarthrodial joints resulting in a loss of the articular cartilage, remodeling of the subchondral bone and inflammatory reaction of the synovium.

Irrespective of the causal factors (genetic, inflammatory, infective, traumatic) the ultimate effect or final common pathway of the disease is full thickness articular cartilage damage with fibrillation, fissures, ulceration and flaking of loose cartilage debris and subsequently joint inflammation (Brandt 2000). Combining the clinical and pathological changes in osteoarthritis, Schnitzer defined osteoarthritis as a disorder of the diarthrodial joints characterized clinically by joint pain and functional limitation, radiographically by osteophytes and joint space narrowing and histologically by alterations in articular cartilage integrity as well as inflammation of the synovium.

Globally, osteoarthritis is the most prevalent chronic musculoskeletal disorder that results in joint pain and between 70%-80% of individuals aged over 55 years have been shown to have radiographic evidence of osteoarthritis although only 10%-20% are symptomatic (Brandt 2000). Although joint pain is the commonest symptom for which most patients with osteoarthritis seek medical attention, the origin of the joint pain is multifactorial involving both the articular and periarticular structures (synovium, subchondral bone, joint capsule, peri-articular muscle, osteophytes and ligaments (Brandt 2000)). However, irrespective of the cause or source of the pain, the ultimate effect is linked to inflammation within and around the aforementioned tissues. The joint pain is initially intermittent when it coincides with the phenomenon called 'flare' (characterized by inflammation of the joint with its sequalea), but over time the pain becomes persistent and deep seated.

The joint inflammation in osteoarthritis (as with inflammation in other organs of the body) will lead to the production of acute phase proteins like C-reactive protein (CRP) along with the release of cytokines and neutrophils. The lysosomes released from the activated neutrophils result in the production of large amounts of free radicals which are harmful to the human body.

Free radicals are unwanted byproducts of normal aerobic cellular metabolism with the potential to damage the various intracellular organelles on which normal cell function depends (Kuo and Schroeder 1995). Antioxidants on the other hand are substances that when present even in low concentrations avidly react with and annihilate free radicals before they (free radicals) can inflict oxidative damage to vital components of the cells including the DNA and the cell membrane.

PATIENTS AND METHOD

Fifty-five subjects (16 males and 39 females), aged between 40-89 years and comprising 35 test subjects randomly selected from the Orthopaedic outpatient clinic with clinical and radiological evidence of osteoarthritis of the knee and 20 age-matched controls with no clinical evidence of osteoarthritis in any joint of the body were recruited into the study which spanned a 3 month period. All the patients were examined by one consultant Orthopaedic surgeon at the surgical outpatient department of the University College Hospital, Ibadan. Ethical approval was sought and obtained from the University of Ibadan/University College Hospital Institutional Review Committee. Informed consent was obtained from all the subjects. Subjects with active infection, hypertension, heavy drinkers, diabetes mellitus, pregnant women, lactating mothers and patients on multivitamin supplementation were excluded.

Routine biodata and anthropometric measurements including body mass index were carried out on all the subjects. 10 milliliters of venous blood was obtained under tourniquet from the antecubital vein from each subject. 5 milliliters (5ml) of the blood sample (sample A) was dispensed into a disposable serum collection vacutainer tubes (to avoid heamolysis) and allowed to clot. The remaining 5 milliliters of blood (sample B) was emptied into vacutainer tubes containing anticoagulant. The blood samples were stored at -20^0C until ready for analysis. For analysis, samples (A and B) were centrifuged for 5 minutes (using a centaur MSE centrifuge machine; Fisons, England) and the serum from the A samples and the plasma from the B samples were analysed within 4 weeks of collection. Total antioxidant status was estimated using the method of Koracevic *et al* (Koracevic *et al* 2001) and the CRP was quantitatively estimated using Humatex CRP kit® (Human GmbH, Germany).

RESULTS

The female to male ratio of the test and control subjects was 6:1. The mean ages, mean BMI and mean TAS levels for test as well as control subjects are as show on table 1. The mean total antioxidant status (TAS) in the test subjects was significantly lower than for the controls (p value <0.05).

Table 1.
Comparison of age, body mass index and total antioxidant status between osteoarthritic patients and controls

Variable	*OA patients (n=35)*	*Controls (n=20)*	*t value*	*p value*
Mean age (in years)	58.8± 12.0	52.3± 7.9	2.21	0.03
Mean BMI (kg/m2)	32.2± 6.9	26.1± 3.4	3.76	0.0004
Mean TAS level (mmol/l)	1.09± 0.38	1.61± 0.09	6.25	0.00009

Nineteen (54.3%) subjects in the test group had a positive agglutination test for C-reactive protein whilst 16 (45.7%) had a negative reaction.

Table 2.
Comparison of total antioxidant status (TAS) in osteoarthritic patients who tested positive for C-reactive protein (CRP) with those who tested negative for CRP

Parameter	*Groups*		*t value*	**p value**
	CRP -ve OA patients (n=16)	CRP +ve OA patients (n=19)		
TAS (mmol/l)	1.23± 0.22	0.96± 0.42	2.32	<0.05

Table 3. Statistical correlation between total antioxidant status (TAS), body mass index (BMI) and age

	TAS	**p value**
Age	-0.361	<0.05
Body Mass Iindex	-0.146	<0.05

Table 2 shows that the total antioxidant status in the test group who tested positive for CRP was significantly lower than for subjects who had a negative reaction (p value <0.05). The Pearson's correlation between the total antioxidant status, body mass index (BMI) and age revealed an inverse correlation between the BMI and TAS (p value <0.05) and also an inverse correlation between age and TAS (p value <0.05) as shown in table 3.

DISCUSSION

Osteoarthritis is a chronic disease of the synovial joint characterized by intermittent episodes of inflammatory reactions (referred to as flares) producing pain, occasional joint swelling, limitation of joint movement and ultimately failure of the joint (Linblade, and Hedford 1987). Following inflammation, neutrophils and other phagocytic cells invade the site of the 'lesion' and the activation of these cells leads to the production of superoxide radicals, hydrogen peroxide, hydrochlorous acid, nitrogen oxide and other potentially damaging free radicals. Although one of the objectives of the free radicals is the destruction of foreign organisms in the vicinity of the inflammatory lesion, the lack of distinction between normal and inflamed cells also leads to the damage of normal cells. This event is deleterious to the body if it is uncontrolled or when it is prolonged as is seen in chronic diseases like osteoarthritis which is characterized by repeated inflammation consequent on irreversible articular cartilage damage.

The total antioxidant status measured in this study using the technique of Koracevic *et al* hinges on the formation of hydroxyl radicals (OH^-) by a Fenton-like reaction using a standardized solution of Fe-EDTA and hydrogen peroxide. The hydroxyl radicals or reactive oxygen species degrade benzoate resulting in the release of thiobarbituric acid reactive substance (TBARS) (Gutteridge JMC *et al* 1990; Yamazaki I and Piette LH 1990). Antioxidants from the serum of the subjects suppress the production of TBARS. This reaction can be measured spectrophotometrically at 532mm wavelength and the inhibition of colour development is defined as the antioxidant status. Therefore, the total antioxidant status measured in this study is not a simple summation of the activities of the various anti-oxidative substances but a dynamic equilibrium that is influenced by the interaction between each serum anti-oxidative constituents. It is also well established that the cooperation of antioxidants in human plasma provides greater protection against attacks by free radicals than individual antioxidants acting alone (Koracevic D *et al* 2001; Wayner DDM *et al* 1987). In osteoarthritis therefore, the value of total antioxidant status is

indicative of the imbalance between free radicals generated in the synovium consequent on inflammatory process and the antioxidant levels in the patients.

The inverse correlation found between body mass index (BMI) and total antioxidant status (TAS) implies that obese subjects are predisposed to diseases mediated by free radicals. A similar inverse correlation between age and TAS by interpretation means that older persons require dietary supplementation of antioxidants which may prevent, delay, or minimize the onset and severity of osteoarthritis and other free radical-mediated diseases.

This study further suggests that the use of antioxidants may complement the anti-inflammatory effect of NSAIDs, in the conservative management of patients with osteoarthritis or other diseases characterized by inflammation. This reinforces the generous use of antioxidants as food supplements in conjunction with dietary sources in developed countries. However, in developing countries like Nigeria, the cost of procuring these supplements may limit their consumption coupled with the dearth of knowledge of their uses. In addition inadvertent depletion of the natural sources of dietary antioxidants due to traditional overcooking of vegetables further shortchanges the Nigerian patient with inflammatory disease like osteoarthritis.

Conclusion

This study, the first in Nigeria shows that Nigerian patients with symptomatic osteoarthritis of the knee tend to be obese (with a higher body mass index) and have a lower total antioxidant status. The positive reaction for C-reactive protein in over half of the symptomatic patients (which is suggestive of active inflammation) and the lower total antioxidant status should act as a guide for the clinicians involved in the treatment of these patients. The need for antioxidant supplementation as a means of preventing osteoarthritis and other free radical-mediated diseases in middle aged adults has been justified.

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