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KNEE OSTEOARTHRITIS INCREASES PAIN PERCEPTION AND ALTERS INTERLEUKINS (6 AND 10) LEVELS IN PATIENTS IN SOUTH-WEST, NIGERIA
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KNEE OSTEOARTHRITIS INCREASES PAIN PERCEPTION AND ALTERS INTERLEUKINS (6 AND 10) LEVELS IN PATIENTS IN SOUTH-WEST, NIGERIA

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ABSTRACT

Background: **Osteoarthritis is a chronic degenerative joint disease which can affect any joint in the body, usually accompanied by pain. The onset and progression of the disease is determined by several factors such as genetic, gender, occupation and ethnicity. There is paucity of information on pain perception and biochemical profile in Osteoarthritis of the knee (KOA) patients in Nigeria.**
Objective: **To assess the pain perception and some biomarkers in osteoarthritic patients in South-west, Nigeria.**
Design: **A retrospective study**
Setting: **Nationally representing South-West region of Nigeria**
Subjects: **A total of sixty human adult subjects were used in this study (Control group, 30 healthy and KOA group, 30 diagnosed with OA)**
Main outcome measures: **All the subjects underwent the Ischemia-induced pain test and blood samples were taken from them for the determination of serum interleukin-6 (IL-6), interleukin-10 (IL-10) and calcitonin gene related peptide (CGRP).**
Results: **The results showed a significantly ($p<0.05$) higher pain threshold and pain tolerance in healthy individuals compared to KOA patients. There was also a significantly ($p<0.05$) elevated level of IL-6 in the serum of KOA patients compared to control (13.0 ± 0.7 vs 20.1±3.2 pg/dl) and a significantly ($p<0.05$) lower level of IL-10 in the serum of OA patients compared to control (14.3± 3.1 vs 4.1 ±0.5 pg/dl).**
Conclusion: **There was no difference in the serum level of CGRP in the control compared with the KOA group. In conclusion, KOA causes decrease in pain tolerance and threshold which is accompanied by alteration in vital biochemical parameters.**

INTRODUCTION

Osteoarthritis (OA) is a chronic irreversible degenerative joint disease characterized by

cartilage degradation which can affect any joints in the body (1-3), but it is mostly reported in weight-bearing joints such as the knee and hip (4-6). It is the commonest chronic disease of human joints and the major risk factors are old age, ethnicity, previous joint trauma, obesity and occupation type (7). The commonest symptoms presented by OA patients are joint pain, joint swelling, locomotive disability, joint stiffness, crepitus. However, joint pain is the commonest complaint that is presented (8).

Radiography is a commonly used tool in the clinics to aid in the diagnosis of OA, but this has limited capability to detect the pathology early enough. Furthermore, there are potential hazards associated with the use of radiography; therefore, there is the need for biochemical approach in the diagnosis and treatment of the ailment. In recent years, there has been a considerable effort to find biochemical markers which could aid in the monitoring of OA. Research has focused the search on two main routes which are firstly, the products of bone and cartilage degradation (9). Secondly, the use of markers of inflammation, which is a shift from the historic view that emphasized that the disease originates primarily from "wear and tear" in the joints only. This second pathway proposed the imbalance involvement of pro-and anti-inflammatory agents, particularly cytokines, in the development and progression of OA and there are evidences from human and animal models of the disease (10-12). Cytokines have also become targets themselves for therapeutic agents in the treatment of OA and they are also equally employed as therapeutic agents (13).

Nigeria is a country with an estimated population of about 196 million (14). OA is a common hospital presentation case according to some hospital-based studies (4,15,16) with an incidence rate of 19.6% among young adults and peaks around late 60's at 39% in this country (17). This does not account for the true prevalence as many incidences in the rural communities "about 51.4% of the total population" are mostly not reported in clinics because many assumed it is just one of the diseases of old age and one, they need to live with (18). There is paucity of information on pain perception and biochemical profile in OA patients in Nigeria as all the previous researches did not account for their pain perception and the role of their pain biomarkers in the management of OA. This study, therefore, assessed pain perception and some biomarkers in knee osteoarthritic patients in South-west, Nigeria.

MATERIALS AND METHODS

Human subjects: Multi-stage sampling technique was used in choosing the subjects used for this study. First stage involved the use of purposive sampling technique in choosing 60 participants for the study based on the recommendation of Voorhis and Morgan (19). The second stage involves the use of stratified sampling technique, giving rise to two strata. Stratum one consists of 30 healthy volunteers were selected in the community as the control group, while stratum two consists 30 volunteer diagnosed knee OA patients, selected consequentially from Orthopedic clinic in Ekiti State University Teaching Hospital as the osteoarthritis group (KOA group).

Protocol: These individuals were older than 45 years, with the mean age of the control group at 56.7±1.02 years and that of OA group at 59.17±1.64 years as seen in Table 1. Knee OA patients were diagnosed using America College of Rheumatology (ACR) criteria. The criteria involve the patients presenting with minimum of three symptoms from i. Age>50; ii. Morning stiffness < 30 minutes; iii. Crepitus on knee motion; iv. Bony tenderness; v, Bony enlargement; vi. No palpable warmth. In

addition, there must be radiographic report (x-ray showing presence of osteophyte) of the affected knee (20). All the subjects were trained and properly briefed about the research and their informed consents were obtained. The distribution as shown in Table 1.

Table 1
Distribution of subjects and their variants

		Control	KOA
Gender	Male	14	16
	Female	16	14
Age	Mean	56.7±1.02 years	59.17±1.64 years
Age range		45-65	48-76

Inclusion criteria for selection of subjects:
The following were the inclusion criteria for subject recruitment into this study:

(a) They must be a known osteoarthritis patient in orthopedic clinic for at least three months.
(b) They must be a known osteoarthritis patient with normal sensation (touch, pain, vibration).

For the control group (normal healthy adult): they must not be on any analgesic medications, not on hospital admission in the last one month, did not had surgery done in the last three months, not diabetic, not suffering from chronic pain syndrome (such as shingles, fibromyalgia and diabetic neuropathic pain)

Willingness of all the patients and healthy volunteers to abide by the rule and protocol of the study, willingness to voluntarily partake in the study and signing of the consent form.

Sub-maximal effort tourniquet test: The ischemic pain testing (sub-maximal effort tourniquet test) was based on the method described by Plesan et al (21). A blood pressure cuff was placed around the non-dominant upper arm of the subject's "on the brachia artery". The cuff pressure was increased to 20mmHg above the subject's systolic blood pressure. With the pressure maintained, subject performed a hand grip exercise on an elastic ball. The subject closes his/her eyes for the entire procedure to minimize distraction and time cues. Subjects were then asked to indicate when they first detected (feel) the pain and when they could no longer tolerate the pain (to a maximum of 300 seconds). Once pain tolerance was reached, the pressure curve was immediately deflated, and endpoints were measured in seconds with the process performed 3 times and average of the readings documented (21).

Pain threshold assessment: The pain threshold is defined as the point between being "about to be painful" and "just became painful" and the time taken for this to occur is recorded in seconds. The process is performed 3 times and the average is documented (21).

Pain tolerance assessment: The pain tolerance is defined as the point at which subjects can no longer withstand the pain and the time taken for this to occur is recorded in seconds. The process is performed 3 times and the average is documented (21).

Biochemical analysis

Determination of serum interleukin 6 (IL6): This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to interleukin 6 (IL6) has been pre-coated onto a microplate. A competitive inhibition reaction was launched between biotin labelled IL6 and unlabelled IL6 (Standards or samples) with the pre-coated antibody specific to IL6. After incubation the

unbound conjugate was washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. The amount of bound HRP conjugate was reverse proportional to the concentration of IL6 in the sample. After addition of the substrate solution, the intensity of colour developed was reversed proportional to the concentration of IL6 in the sample.

Determination of serum interleukin 10 (IL10): This assay also employs the competitive inhibition enzyme immunoassay technique. Monoclonal antibody specific to interleukin 10 (IL10) was pre-coated onto a microplate following which a competitive inhibition reaction was launched between biotin labeled IL10 and unlabeled IL10 (Standards or samples) with the pre-coated antibody. After incubation, the unbound conjugate was washed off, and avidin conjugated to HRP was added to each microplate well and incubated. The amount of bound HRP conjugate inversely corresponds with the concentration of IL10 in the sample. The substrate solution was added to each microplate well leading to coloration of the solution. The intensity of colour developed was reversed proportional to the concentration of IL10 in the sample.

Determination of serum calcitonin gene-related peptide: Likewise, this assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to calcitonin gene-related peptide (CGRP) has been pre-coated onto a microplate in which a competitive inhibition reaction was launched between biotin labeled CGRP and unlabeled CGRP (Standards or samples) with the pre-coated antibody. Following incubation, the unbound conjugate was washed off, then avidin conjugated to HRP was added to each microplate well and incubated. The amount of bound HRP conjugate was also inversely proportional to the concentration of CGRP in the sample. Finally, we added the substrate solution and the intensity of color developed was reversed proportional to the concentration of CGRP in the sample

Statistical analysis

All data were expressed as the Mean ± SEM. Tests for homogeneity of the varied intervention carried out by using Independent-Samples T test from SPSS version 20 software with the level of significance set at $p < 0.05$.

All procedures were performed in the Orthopaedic Clinic of Ekiti State University Teaching Hospital according to the ethical guidelines of human subjects, which are, respects for persons, justice and beneficence. All subjects signed an informed consent after the purpose, risks, clinical benefits and results usage of the study were fully discussed with them all. *Approval (Protocol number):* EKSUTH/A67/2016/12/005) was obtained from the Research and Ethical Review Committee of the Ekiti State University Teaching Hospital, Ado Ekiti, Ekiti State, Nigeria.

RESULTS

Effect of knee osteoarthritis (KOA) on pain threshold: Pain threshold among the control group and KOA patients are shown in figure 1. There is significant lower pain threshold in KOA group (22.43±1.16 seconds) compared to the control group (30.83±0.09 seconds) with the $p<0.03$.

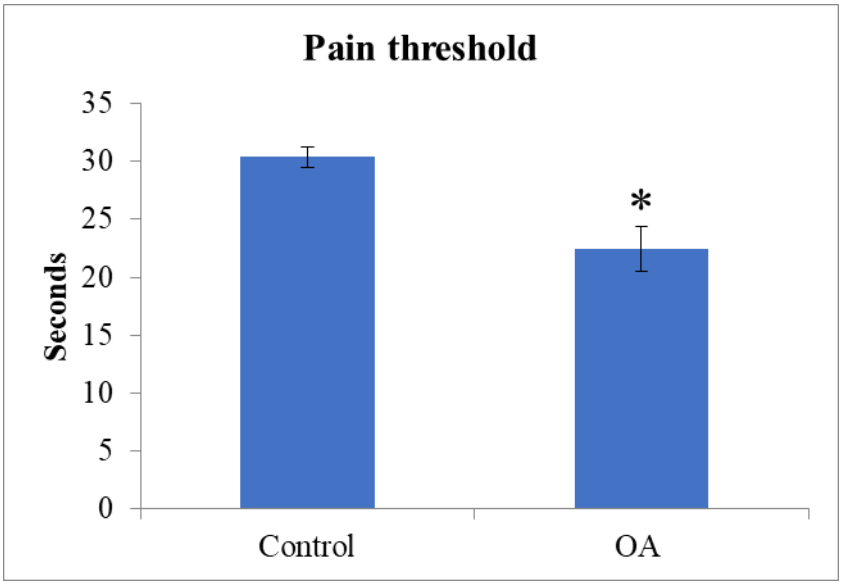

Figure 1 shows the pain threshold among the control group and osteoarthritis group. Pain threshold was *significantly (p<0.03) reduced in OA group compared to the control. Values are expressed in Mean± SEM.

Effect of knee osteoarthritis (KOA) on pain tolerance: Pain tolerance among the control group and KOA patients are shown in figure 2. There is significant lower pain threshold in KOA group (43.87±0.91 seconds) compared to the control group (61.8±0.09 seconds) with the p<0.05.

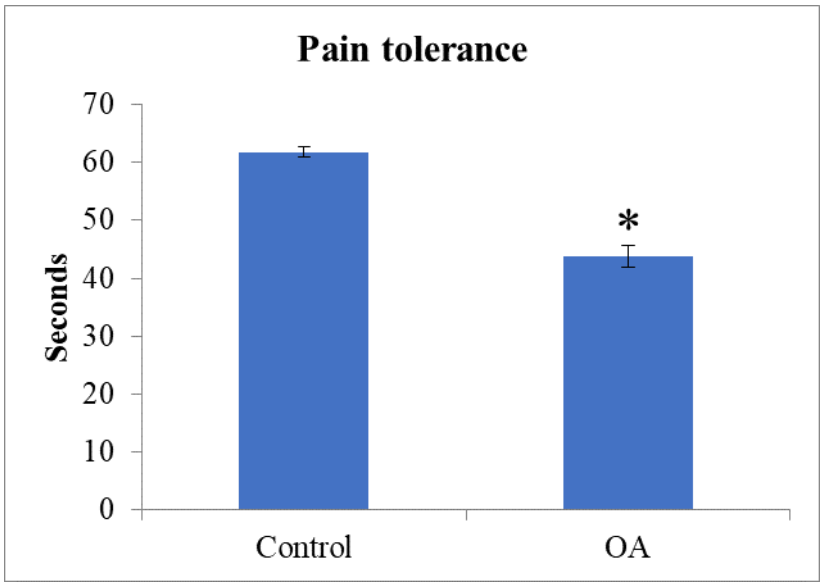

Figure 2 shows the pain tolerance among the control and osteoarthritis groups. Pain tolerance was * significantly (p<0.05) reduced in OA group compared to the control. Values are expressed in Mean± SEM.

Effect of knee osteoarthritis (KOA) on serum level of IL-6: Figure 3 shows the serum level of IL-6 among the control and KOA patients. There is significant higher serum level of IL-6 in KOA (20.1±1.2 pg/dl) compared to the control (13.0±0.7 pg/dl) with the $p<0.05$.

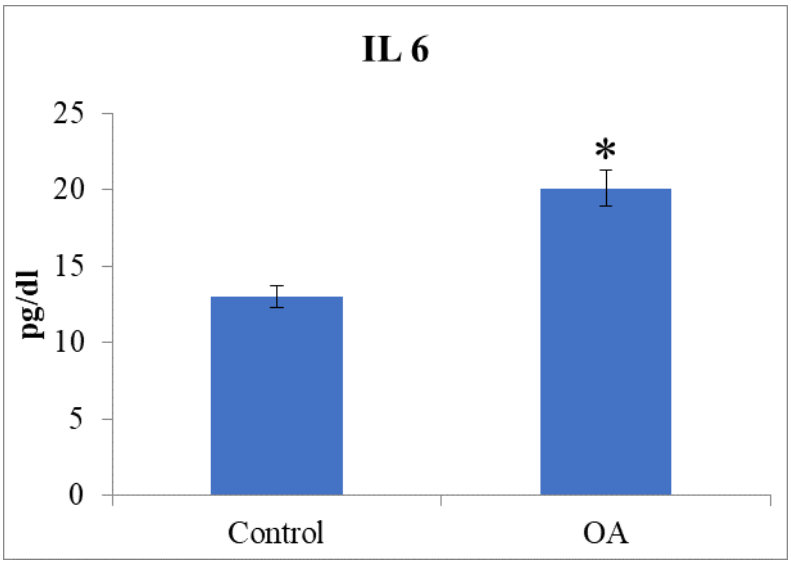

Figure 3 shows the serum level of Interleukin-6 in control group and in osteoarthritis group. Serum IL-6 level in OA group was *significantly ($p<0.05$) higher compared to the control. Values are expressed in Mean± SEM.

Effect of knee osteoarthritis (KOA) on serum level of IL-10: Figure 4 shows the serum level of IL-10 among the control and KOA patients. There is significant lower serum level of IL-10 in KOA (4.1±0.5 pg/dl) compared to the control (14.3±3.1 pg/dl) with the $p<0.01$.

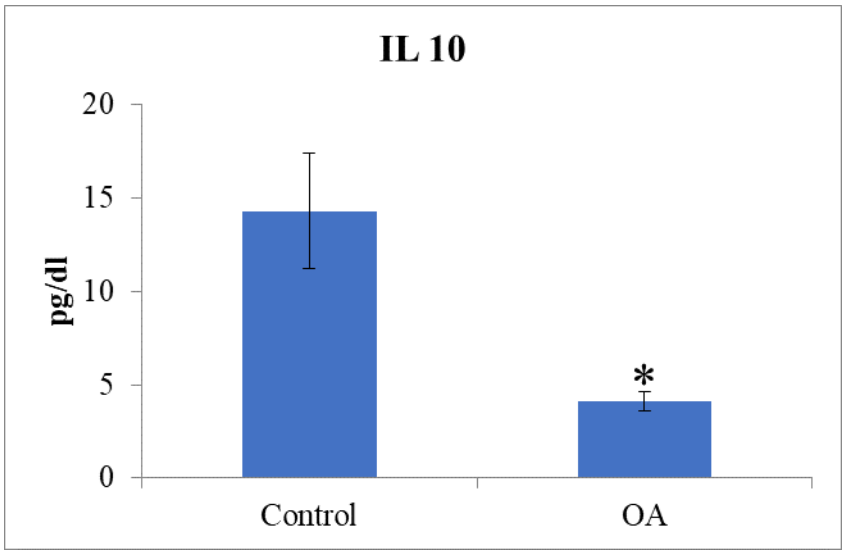

Figure 4 shows the serum level of Interleukin-10 in control group and in osteoarthritis group. Serum IL-10 level in OA group was *significantly ($p<0.01$) reduced compared to the control. Values are expressed in Mean± SEM.

Effect of knee osteoarthritis (KOA) on serum level of CGRP: Figure 5 shows the serum level of CGRP among the control and KOA patients. There is no significant difference in the level of serum CGRP in KOA (146.07±4.26 pg/dl) compared to the control (142.93±5.1 pg/dl) with the p>0.05.

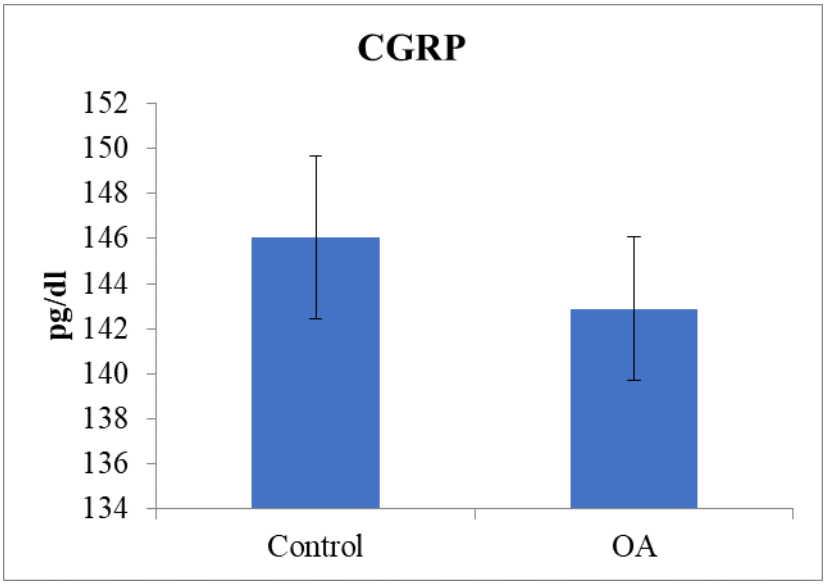

Figure 5 shows the serum level of CGRP in control group and in osteoarthritis group. Serum CGRP level in OA group was not significantly (p>0.05) reduced compared to the control. Values are expressed in Mean± SEM.

DISCUSSION

Pain perception and biomarkers in knee osteoarthritis patients in South-west, Nigeria was assessed in this study with the aim of providing information on pain perception and biochemical profile in these patients compared with their healthy peers. The results showed significantly lowered pain threshold and pain tolerance in OA group compared to the control group, indicating an increase in their pain sensitivity. These observations are similar to previous finding characterized by hyperalgesia and spontaneous pain noticed in OA patients (22,23). The decrease in pain threshold and tolerance ultimately leads to the reduction in the quality of life of these patients.

This study further showed that serum level of IL6 was increased in OA patients. This is consistence with the report of Livshit et al in 2009 (24), first reported a significantly higher serum concentration of IL 6 OA and other subsequent reports (24-26). IL-6 is a glycoprotein consisting of 184 amino acid residues (27). They have been implicated in the pathogenesis and progression of joint disease usually in response to IL-1β and TNFα (6). They are produced by chondrocytes, adipocytes and osteoblasts (28-30), which might have accounted for the high concentration of IL-6 found in both the serum and the synovial fluid of affected knee (31). Stannus et al (32) reported that the serum concentration of IL-6 is directly proportional to the level of the severity of OA as revealed by the radiograph of the

affected knee joints. The relationship between the pain behaviour noticed in these patients and the serum level of IL-6 is best explained in the context that IL-6 influences transduction, conduction, and transmission of the nociceptive signals (33). In turn, IL-6 formation is induced by raised shear stress and other catabolic stimuli such as cytokines (34).

The study also showed a significant reduction of serum level of IL-10 in OA group when compared to the control group, results within the normal range (35). This is different from the report of Imamura et al (26), who reported a higher serum level in OA patients compared to the control in a study done in Sao Paulo, Brazil. However, a more recent work on rat study reported a lower serum concentration of IL 10 compared to the control (36). Interleukin-10 is a known anti-inflammatory cytokine that is structurally related to interferons (37) and it is synthesised by the chondrocytes (38). It stimulates the synthesis of proteoglycan by promoting the synthesis of aggrecan and type II collagen (39). Interleukin-10 inhibit pro-inflammatory cytokines like IL-1β, IL 6 and TNFα and also, as well as antagonising the apoptosis of chondrocytes, preventing cartilage degradation (40). The role of exercise in the management of Knee OA has been linked to an increase in intra-articular concentration of IL 10 (38.41). The chondro-protective and anti-inflammatory property of IL-10 is reduced drastically as seen in this study, making it a potential therapeutic route to abate the progression of OA.

The CGRP level was not significantly altered between the two groups showing that it might not be the pathway by which OA manifest. CGRP is one of the most potent micro-vascular vasodilators (42) within the human body. It mediates its actions by acting on cAMP pathways to cause the opening of ATP-sensitive potassium (k) channels, resulting in vasodilatation (43). Our result suggests that OA is more of an immunological pathology than inflammatory condition, so CGRP does not serve a prognostic role as the other two cytokines understudied in this study. This study had some limitations like, our inability to assay the intra-articular fluid for cytokines and enlarging our sample size, due to the budget of the study.

CONCLUSION

Osteoarthritis is associated with increased pain perception, evidenced by their poor pain sensation control. The plasma concentrations of interleukins 6 and 10 are altered in these patients; therefore, early detection and possible correction of these derangements may prevent progression of this pathology.

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44. Confirm observance of maximum references as well as total word count