EFFECT OF 5'-NUCLEOTIDASE AND ADENOSINE ON PATHOGENESIS DESTRUCTION OF THE KNEE AND HIP TISSUES IN PATIENTS WITH OSTEOARTHRITIS

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ABSTRACT

Osteoarthritis (OA) is a long-term chronic illness marked by the degeneration of cartilage in joints, resulting in bones rubbing together, causing stiffness, discomfort, and restricted movement. The illness most often affects the knees, hands, feet, and spine, with shoulder and hip joints being quite prevalent. While OA is linked to aging, it is also linked to several risk factors, such as obesity, inactivity, genetic predisposition, bone density, occupational damage, trauma, and gender. The present study focuses on 5'-nucleotidase (NTD) and Adenosine changes in the knee and hip of patients with OA. Tissue samples were obtained from 50 OA patients (25 knees and 25 hips) during knee and hip replacement for Enzyme histochemical study, and collection of blood samples from every 50 patients and 25 control for Adenosine. The tissue and blood samples were collected during the period October 2019 to April 2020. The Enzyme histochemical study of knee OA in femoral bone showed severe activity of NTD in osteocytes while in the fibrocartilage of knee OA patients demonstrate the moderate activity of the enzyme in fibrocytes. In the femoral bone of hip OA patients showed the moderate activity of NTD enzyme in osteocytes while in the fibrocartilage of hip OA patients demonstrate the moderate activity of NTD enzyme in fibrocytes. However, the hip ligament showed up for OA patients severe activity of NTD enzyme in fibrocytes. Our results showed a highly significant elevation in Adenosine there was a significant difference between control and tested groups. On the other hand, there was a significant difference between tested groups in the patients.

Keywords: Osteoarthritis, Knee, Hip, 5'-nucleotidase, Adenosine.

1. INTRODUCTION

Osteoarthritis (OA) is a group of heterogeneous conditions that lead to joint signs and symptoms associated with defective articular cartilage integrity, in addition to the associated changes in the underlying bone at the edges of the joint (1), also known as "wear" arthritis. This is a progressive degenerative joint disease that primarily affects articular cartilage, but it also suffers from other structures in the joint. Symptoms of OA include pain joint stiffness and joint swelling. Reduced range of motion, and crepitus (2). The knee joint is the largest and most stressful synovial joint. The musculoskeletal system of the human body supports the body weight and facilitates locomotion. The knee joint is the largest and most heavily loaded synovial joint in the musculoskeletal system of the human body, which supports the body weight and facilitates locomotion (3). The hip joint, also known as the acetabular femoral joint, is the ball-and-socket joint. Joint is formed by the joint between the pelvic acetabulum and the femoral head. It plays a major role in supporting weight in both static and dynamic positions (4). Due to the uniqueness of the hips, it is very stable as it is designed primarily with weight in mind. The weight of the human body is through the hip joint lower limbs (5). 5'Nucleotidase (NTD) (EC3.1.3.5) also known as 5'-ribonucleoside phosphohydrolase has been identified as the enzyme responsible for the dephosphorylation of extracellular mononucleotides in a large variety of cellular systems and catalyzes the extracellular conversion of 5'-AMP to adenosine (7). Adenosine is a purine
nucleoside that has been described as an endogenous and pervasive regulator of a variety of tissue and cell functions. Adenosine is formed in the extracellular space by a sequence of external enzymes degrading Adenosine triphosphate (ATP). Adenosine mainly exerts its effects by interacting with four G protein-coupled receptors (GPCRs). Adenosine receptors (ARs) A1, A2A, A2B, and A3 are found in a variety of cells and tissues in the body. These receptors are expressed on immune system cells, and they react to the regulatory effects of adenosine in an inflammatory context 

II. MATERIALS AND METHODS

The study included 50 patients with OA, who were obtained from Nursing home hospital and Ghazy Al-Hariri hospital for surgical specialties /Medical city during the period from October 2019 to April 2020. In addition 25 samples as a control for the Adenosine test.

The tissue specimens (bone, cartilage, ligament) were obtained during operations involving the total knee and hip replacement, from 50 (25 knees and 25 hips) patients with OA, which is diagnosed by specializes, doctor. Tissues were fixed and dehydrated in a mix of absolute alcohol and cold acetone for different periods and embedded in paraffin wax. NTD demonstrated in paraffin sections (5μm thick) as described by

From every 50 patients and 25 control, venous blood samples (7 ml) were pulled from all individuals participating in this study; these samples were placed in a clean test tube and left for 15 minutes at room temperature for coagulation. The serum was extracted by placing samples in a centrifuge at 3000 r.p.m. then serum was subdivided and placed into new and clean test tubes (500 microliters of serum in each test tube) and kept at a temperature of – 20º C until utilized. To demonstrate the Adenosine level in serum with the commercially available kit( Abbkine) in Chin.

The Statistical Analysis

The data collected were subjected to a one-way variance analysis (ANOVA) test to compare different groups with each other. Effects were expressed as mean +standard error(SE) and values of p>0.05 were considered to be statistically non-significant while p<0.05 was considered to be significantly different. The statistical analysis was carried out by Statistical Package for the Social Sciences (SPSS) version (v 20).

III. RESULTS AND DISCUSSION

Enzyme Histochemical Study

A) The-5'-Nucleotidase (NTD) In Knee Tissues

The femoral bone of knee OA patients showed the severe activity of NTD enzyme in osteocytes (Fig-1A) while the fibrocartilage of knee OA patients demonstrated the moderate activity of NTD enzyme in fibrocytes (Fig-1B). However, the knee ligament showed up for OA patients severe activity of NTD enzyme in fibrocytes (Fig-2).

Figure 1. Cross-section in the knee of OA patients showed: A) compact femoral bone of knee patients showing the severe activity of NTD enzyme in osteocytes (black color)(X10). B) the fibrocartilage of knee patient demonstrate the moderate activity of NTD enzyme in fibrocytes (black color) (Large figure X10, small figure X40).
Figure 2. Cross-section in the ligament of knee OA patient demonstrate the severe activity of NTD enzyme in fibrocytes (black color) (Large figure X10, small figure X40).

B) The-5'-Nucleotidase (NTD) In Hip Tissues

The femoral bone of hip OA patient showed the moderate activity of NTD enzyme in osteocytes (Fig -3A) while in the fibrocartilage of hip OA patient demonstrated the moderate activity of NTD enzyme in fibrocytes (Fig -3B). However, the hip ligament showed severe activity of NTD enzyme in fibrocytes (Fig- 4).

Figure 3. Cross-section in the hip of OA patients showed: A) the moderate activity of NTD enzyme in osteocytes (black color) (X10). B) demonstrate the moderate activity of NTD enzyme in fibrocytes (black color) (X10).

Figure 4. Cross-section in the ligament of hip OA patient demonstrate the severe activity of NTD enzyme in fibrocytes (black color) (Large figure X10, small figure X40).

Adenosine Level

The result of the present study showed a highly significant (p<0.05) increased level of Adenosine in the serum of knee and hip OA patients as compared with the control group. Table (1) showed Adenosine levels in the knee and hip OA patients were 318.20±44.2 ng/ml and 382.18±53.7 ng/ml respectively, while in the control group was 66.52±1.8 ng/ml. On the other hand, there was a significant difference (p < 0.05) between the knee and hip OA patients.
The results showed a relationship between NTD enzyme and adenosine in the knee and hip OA patients. Increased production of adenosine, a factor known to induce cell death in some cell types. The increase of NTD in serum of OA patients is related to its release from damaged cells. The enzyme plays a key role in the metabolism of nucleotides⁶. In addition, NTD is implicated in pathophysiological conditions through its control of the extracellular adenosine level⁷. In the present study, an increase in the activity of NTD in patients with OA in the knee and hip. However, it is found that the activity of NTD increases in OA would result in increased production of Adenosine, a factor known to induce cell death in some cell types. NTD activity is low in circulating monocytes, increases markedly upon their differentiation to tissue macrophages, and subsequently diminishes during macrophage activation⁸.

Adenosine

The Immune responses are aggravated and tissue damage is compounded in the lack of functional adenosine signaling at sites of infection, inflammation, and injury. Extracellular adenosine concentrations differ due to physiologic and pathologic stimuli such as hypoxia and inflammation⁹. Adenosine and its receptor A2AR play a crucial role in maintaining cartilage and joint homeostasis by inhibiting cartilage degradation and promoting cartilage matrix formation by chondrocytes⁰. The primary source of adenosine in the extracellular space is ATP, which is transferred into the extracellular space and hydrolyzed by exoenzymes to adenosine. ATP levels in chondrocytes and other cell types decrease with age, most likely due to mitochondrial dysfunction, and lower levels of ATP are transferred into the extracellular space, the resulting diminution of A2AR stimulation permits chondrocyte hypertrophy and expression of proteins, like MMPs, that contribute to the development of OA. In contrast, in inflamed and injured cartilage cytokines enhance both A2AR expression and function thus magnifying the functional effects of A2AR stimulation¹¹,¹².

IV. DISCUSSION

The results showed a relationship between NTD enzyme and adenosine in the knee and hip OA patients. The enzyme histochemical changes showed severe and moderate activity of NTD enzymes in the knee and hip OA patients, which caused increased production of adenosine. Adenosine serum level has shown a highly significant increase in OA patients. Increased production of adenosine, a factor known to induce cell death in some cell types.

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