Alteration in Serum Levels of Calcium in the Patients of Knee Osteoarthritis

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Abstract: *Osteoarthritis is the most common type of arthritis affecting mostly the weight bearing joints. Cytokines are glycoprotein secreted by the different cells and responsible for immune response. Proinflammatory cytokines including IL-6 are predominately secreted by macrophages & other cells and they are involved in up regulation of inflammatory response. IL-6 may induce expression of proteolytic enzymes which cause cartilage damage and in turn osteoarthritis. The present study was conducted in the Department of Biochemistry in collaboration with Department of Orthopedics of Pt. B.D. Sharma Post Graduate Institute of Medical Science, Rohtak. Forty patients with knee osteoarthritis in the age group of 30-60 years were enrolled for the study. Forty healthy volunteers of the same age group were taken as control. After taking written consent, Five mL of venous blood from the patients and controls was taken from antecubital vein in a red capped vaccutainer under aseptic conditions. Estimation of serum calcium was done on the same day by standard autoanalyzer kit method and was expressed as mean±SEM. The serum levels of calcium were found higher in cases as compared to the controls (p<0.001).*

Keywords: Knee, Osteoarthritis, Cytokines, Interleukins

1. Introduction

Osteoarthritis is a degenerative joint disorder of the weight bearing joints like knee and hip. It is the most common cause of joint paint. OA is due to the progressive cellular and changes in all tissues of the joint. Ageing is the most common cause of the OA. It affects joint cartilage, Subchondral bone as well as synovium [1]. The ageing cells alter mitochondrial function due to the oxidative stress which lead to the cell death. In ageing cells, there is down regulation of the transforming growth factor- β (TGF- β) and chondrocytes instead of matrix synthesis shift towards catabolic side and matrix metalloproteinases (MMPs) expression. Obesity is another etiological factor in the development of OA. It is the most promising problem in recent years due to the modification in the life style of people. Adipokines are adipose tissues derived cytokines which are relased by adipocytes in obese persons. In obese person, the levels of pro- inflammatory cytokines are found elevated. This lead to NB-kb pathway activation which ultimately lead to initiation of catabolic process in the chondrocytes & ECM degradation [2,3]. Sport injury is another etiological factor in the development of OA, especially in young population. It is the most common cause of OA in young and middle aged population [4, 5].

In recent years, researchers have demonstrated the role of inflammation in the development and progression of knee OA. Inflammation may be caused by cytokine- induced mitogen activated protein kinase (MAP), NF-kB activation and oxidative phosphorylation. It involves the entire joint including cartilage, synovium and subchondral bone.[6] It is well established that the low grade inflammation found in OA lead to further diseases development and progression.

Although it is yet not clear that at what extent inflammation can cause cartilage demage. It plays a central role in diverse host defense mechanisms such as the immune response, hematopoiesis and acute phase reactions [7]. IL-6 is a lymphokine produced by antigen or mitogenic activated T-cells, fibroblasts, macrophages and other cells that serve as a differentiation factor for B-cells & thymocytes which stimulates immunoglobulin production [8, 9].

1.1 Cytokines

Cytokines are low molecular weight proteins and their molecules carry same polypeptide fold with four α- helical bundles [10]. They are produced from T_H1 cells, T_H2 cells, T_H17 cells or T_{Reg} cells. They can be classified as: Chemokines i.e. these cytokines are chemotactic for inflammatory cells, lymphokines i.e these cytokines are produced by T-cells and are responsible for immune response, pro- inflammatory cytokines i.e responsible for inflammation and ant-inflammatory i.e. cytokines which oppose the inflammation [11,12].Cytokines bind with their receptors and produce cellular & humoral response, initiation of inflammatory response, control of hematopoiesis, cellular proliferation & differentiation [13,14]. Cytokines often produce in cascade and acts in paracrine (Acts on the cells which produce them), autocrine (Acts on nearby cells) or endocrine manner (Acts on cells at distance after being carried in blood or tissue fluids) [15, 16].

Pro- inflammatory cytokines are responsible for the inflammation and includes: IL-1, IL-6, IL-8, IL-12, IL-15, IL-17, IL-18, tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ), oncostatin M (OSM), granulocyte-macrophage colony-stimulating factor (GM-CSF) and macrophage colony-

stimulating factor (M-CSF). IL-6 is a glycoprotein consisting of 184 amino acid residues.[17,18] IL-6 exerts its biological activities through two molecules: IL-6R (also known as IL-6Rα, gp80 or CD126) and gp130 (also referred to as IL-6Rβ or CD130). It is most potent proinflammatory cytokine. Proinflammatory cytokines stimulate both cellular and humoral inflammatory response. Anti-inflammatory cytokines oppose the inflammation and includes: IL-4, IL-10, IL-11, IL-13 and tumor necrosis factor-β (TNF-β). They inhibit the pro-inflammatory cytokines production. The most common method used to diagnose the knee OA is radiography. Since OA may take years to develop, it is an attractive idea to develop the noval biomarkers which can predict the diseases before the radiological changes appears [19, 20].

2. Materials and Methods

The present study was conducted in the Department of Biochemistry in collaboration with Department of Orthopedics of Pt. B.D. Sharma Post Graduate Institute of Medical Science, Rohtak. The present study was approved and ethical clearance was taken from the ethical committee. Written consents were taken from all patients and controls.

2.1 Study Population

Forty patients with knee osteoarthritis in the age group of 30-60 years were enrolled for the study. Forty healthy volunteers of the same age group were taken as control. All volunteers fulfilled inclusion and exclusion criteria. Patients of knee osteoarthritis suggestive of Diabetes, atherosclerosis, depression, systemic lupus erythematous, any malignancy and other chronic diseases were excluded from this study.

2.2 Sample collection

After getting the written consent from cases and controls, detailed history was taken and recorded in their respective performa. Five mL of venous blood from the patients and controls was taken from antecubital vein in a red capped vaccutainer under aseptic conditions after an informed written consent. Serum was separated for the estimation of calcium by centrifugation at 2000 rpm for 10 minutes after clotting.

3. Statistical analysis

The data was compiled and analysed by unpaired t-test using SPSS 20 version. *P* values less than 0.05 (2-tailed) were considered significant and *P* values less than 0.001 (2-tailed) were considered highly significant.

4. Results

The baseline clinical data of cases and controls are presented in Table-1. Mean age of cases (47±1.46 years) and controls (44.15±1.27 years) were compared which were found statistically not significant (p<0.05). The BMI of cases and controls were (Mean±SEM) 24.69±0.53 & 23.28±0.81

respectively which were also found statistically not significant (p<0.05). In our study, male and females were equally distributed as cases & controls (Table-2). So, the cases and controls were similar with regard to age, proportion of sex.

Table 1: Comparison of age and BMI (body mass index) between the knee osteoarthritis (OA) and healthy control groups (Mean±SEM)

| Parameter	Cases (n=40)	Controls (n=40)	P value
Age (year)	47±1.46	44.18±1.27	P<0.05
BMI (kg/m²)	23.28±0.81	24.69±0.53	P<0.05

Table 2: Sex wise distribution of cases and controls

Cases		Controls	
Female	Male	Female	Male
33	7	33	7

The mean serum levels in cases and controls are presented in Table-3 and represented in figure-1. The mean serum levels of calcium in cases & controls were (Mean±SEM) 9.56±0.16 (mg/dL) & 10.5±0.14 (mg/dL) respectively which were found statistically highly significant (p<0.001).

Table 3: Serum levels of Calcium in cases & controls (Mean±SEM)

Parameters	Cases	Controls	P value
Serum Calcium (mg/dL)	9.56±0.16	10.5±0.14	P<0.001
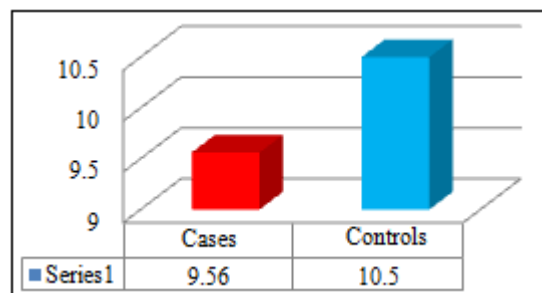
Figure 1: Serum levels of Vitamin D in cases and controls (Mean±SEM)

5. Discussion

Calcium is involved in matrix synthesis, cytoskeletal remodeling, cell hyperpolarization and cell death. It is important for maintaining calcium homeostasis and cell viability. Chondrocytes functions may be impaired under conditions of calcium insufficiency. Calcium (Ca) and inorganic phosphate (Pi) are the two main constituents of hydroxyapatite. The initial step of Ca-Pi crystal nucleation takes place within matrix which leads to the formation of hydroxyapatite crystal. Mineralization of the articular cartilage by Ca-Pi crystal was strongly associated with OA development [21]. In our study, A strong association between the serum levels of calcium were found between the patients of knee OA and controls (p<0.001). Similar findings were reported by Li et al. They reported inverse relationship between serum calcium concentration and radiographic OA of

the knee [. Even though there is no direct evidence found that calcium plays a role in the pathogenesis of OA, these studies support that calcium is involved in physiological and pathological processes of chondrocyte. Chondrocytes functions may be impaired under conditions of calcium insufficiency [22]. We have some limitation of our study also. There is lack of radiographic evaluation of the control subjects. Although during clinical evaluation of the controls, absence of pain and no other complains in controls were observed. Secondly, the study is performed on smaller sample size. The results may be explored widely if performed on a larger sample size. So, further large scale studies are needed to assess the validity and efficacy of these biomarkers.

6. Conclusion

In conclusion, the present study found an inverse relationship between the serum levels of calcium and knee osteoarthritis. Deficiency of calcium may be responsible for the development of knee osteoarthritis. Normal or high levels of calcium may be protective and may prevent the development of knee osteoarthritis.

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