ORIGINAL ARTICLE

A COMPARISON OF SERUM NUCLEAR FACTOR ERYTHROID 2-RELATED FACTOR 2 IN DIABETIC AND NON-DIABETIC PATIENTS WITH OSTEOARTHRITIS

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Background: Osteoarthritis (OA) or inflammation of joints is a chronic degenerative and irreversible joint disorder. Oxidative stress in chronic inflammation contributes to osteoarthritis. Nuclear factor erythroid 2-related factor 2 (Nrf2) signalling pathway have an inhibitory role in oxidative stress and inflammation, as significant interaction exists between transcriptional pathways involving Nrf2 and NF-κB which is related to immune response in body. Diabetes mellitus being a pro inflammatory condition, has more oxidative damage due to the effects of hyperglycaemia. This study evaluated serum Nrf2 expression levels in osteoarthritis patients in the presence and absence of hyperglycaemia in order to find out the magnitude of anti-oxidative response, whether Nrf2 expression levels increases or decreases in oxidative stress environment comparatively. Objectives of this study were to evaluate Nrf2 serum expression levels in osteoarthritis patients by ELISA and to compare Nrf2 serum circulatory levels between diabetic osteoarthritis patients' group and non-diabetic osteoarthritis patients' group. **Method:** This comparative cross-sectional study was conducted from February 01, 2023 to 31 January, 2024 at department of Biochemistry, Nowshera medical college (NMC), Nowshera and Medicine/Orthopaedic OPD of Qazi Husain Ahmad Medical complex (QHAMC), Nowshera. This study included 44 male and female osteoarthritis patients of ≥50 years old that were further divided into a diabetic and non-diabetic group on the basis of hyperglycaemia. NRF2 levels were measured using ELISA. **Results:** Blood circulatory levels of NRF2 were significantly higher in non-diabetic patients of osteoarthritis **Conclusion:** Nrf2 levels are lower in diabetic patients of osteoarthritis as compared to non-diabetic patients of osteoarthritis.

Keywords: Nuclear factor erythroid 2-related factor 2 (Nrf2); Peripheral blood; Oxidative stress; Antioxidants

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INTRODUCTION

Osteoarthritis (OA) is a type of degenerative joint disease. It is recognized as the fourth most common cause of disability globally, Presently, OA affects around 237 million individuals worldwide, or 3.3% of the total population, and is the most prevalent type of arthritis. Incidence increases with age. About 10% of males and 18% of females over 60 are affected.[1] The causes include genetic factors, abnormal development of the joint or limb, and history of joint injury. Low-grade inflammatory processes and mechanical stress on the joint are thought to be the causes of osteoarthritis. Muscle atrophy may happen because pain may make it harder to exercise in advanced joint disease.[1–3] Osteoarthritis (OA) is most commonly characterized by joint stiffness and pain. The knee and hip joints, the joints in the neck and lower back, distal and proximal and distal phalangeal joints are most frequently affected. In contrast to several other forms of arthritis, this one affects solely the joints and not the internal organs.[3–6] Diagnosis is based on clinical history and further procedures, such as medical imaging. For overweight, weight loss may be helpful. In addition to NSAIDs like ibuprofen or naproxen, Paracetamol (acetaminophen) is sometimes used as analgesics. Inadequate knowledge about the risks of addiction and other negative effects, along with the benefits of using opioids, makes long-term use of these drugs not advised. If the disability persists after receiving alternative therapies, joint replacement surgery can be considered.[4] Previous study has shown a strong correlation between oxidative stress and reactive oxygen species (ROS) and the advancement of osteoarthritis (OA). Changes in the morphological, biochemical, molecular, and biomechanical properties of extracellular matrix (ECM) cause softness, articular cartilage loss, synovitis, sclerosis of the subchondral bone, the development of osteophytes, and subchondral cysts.[7,8]

Many processes lead to oxidative stress in diabetes, including the excessive production of oxygen radicals from the autoxidation of glucose, the glycation of antioxidant enzymes, and the formation of glycated proteins, which limits the capacity of antioxidants to detoxify free radicals. Diabetes itself and hyperglycaemia contributes more to oxidative stress and damage in comparison to oxidative stress and damage of senility. Oxidative stress breaks down extracellular components as well as cellular membranes and nucleic acids in OA. This results in altered biological activity, alterations in protein structure, and a build-up of damaged proteins in the tissue.[9] Nfk2 is the primary regulator of antioxidant activity in a cell.

There are currently no proven treatment options for OA. In clinical settings, non-steroidal anti-inflammatory medicines (NSAIDs) have been the most often prescribed treatment for osteoarthritis (OA). Naturally occurring NRF2 up-regulators can be used to control oxidative stress, chronic inflammatory responses, and other aberrant cellular signalling pathways. Nevertheless, there aren't many potent medications that can stop or slow the advancement of diabetes and osteoarthritis (OA). Thus, it is essential to comprehend the antioxidant transcriptional regulator Nrf2's expression levels.

MATERIAL AND METHODS

The comparative cross-sectional study was conducted from February 01, 2023 to 31 January, 2024 at department of Biochemistry, Nowshera medical college (NMC), Nowshera after ethical approval of institutional ethical review committee. After written, informed consent, a total of 330 Osteoarthritis patients were interviewed and only 44 were recruited through Non-probability convenient sampling from Medicine/Orthopaedic OPD of Qazi Husain Ahmad Medical complex (QHAMC), Nowshera.

Sample size of 44 was calculated using openEpi software with a confidence interval (95%) and power of (80%).[9] *p*-value of less than (0.05) was taken as significant.

A total of 44 Osteoarthritis patients of both male and female gender, age ≥ 50 years were further divided into two groups of 22 each on the basis of hyperglycaemia. Group-I having diabetes mellitus for at least 10 years and having no other cause for osteoarthritis except old age and diabetes. Group-II osteoarthritis patients having no other cause for osteoarthritis except old age.

Those who refused to give consent for the study and patients with Septic Arthritis, Traumatic Arthritis, Juvenile Arthritis, Rheumatoid Arthritis etc. were excluded from the study on the basis of history and examination. Detail history and medical examination of included subjects was done. Diagnosis of osteoarthritis was made clinically by supported by investigations. Hba1c test where required was also performed.

Blood samples (2 cc) were taken using aseptic measures and was immediately transferred into microfuge tubes for serum separation. Serum was stored at -80 c after labelling each sample. Nrf2 levels in the subject's blood samples were measured by ELISA (ray bio tech; Id number ELH Nrf2-1Q16236) at department of biochemistry, NMC. Data collected was entered, analyzed and interpreted with the help of MS Excel and SPSS. Before performing statistical analysis, data was checked for normality, skewness and kurtosis which reflected that our data is normally distributed. Descriptive parameters are presented in the form of mean and standard deviation. Comparison of Nrf2 blood expression levels (pg/mL) between the two groups was done the help of student T-Test for comparison among two groups. *p*-value of less than 0.05 was considered as significant.

RESULTS

In both groups male and female gender ratio was equal (11 male and 11 females per group). There was no significant difference in the age of both groups. BMI was also found non-significant when compared between the two groups (*p* value =0.517).

Blood NRF2 expression levels in pg/mL were evaluated, recorded and compared between diabetic and non-diabetic osteoarthritis groups as follows. In our results we have mean values of blood NRF2 expression levels in group I diabetic osteoarthritis as 1.306±0.800 and in group II non-diabetic osteoarthritis the mean blood circulatory expression levels of NRF2 is 2.309±1.290 (*p* value = 0.03 which is statistically significant).

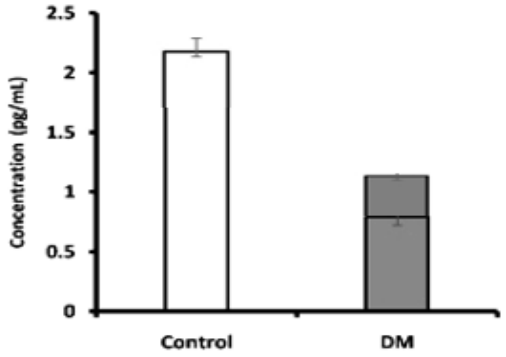

Figure-1: Comparison of Nrf2 Blood Circulatory Levels between Group I (Diabetic Osteoarthritis and Group II (Non-diabetic Osteoarthritis)

Table-1: Comparison of study parameters in diabetic & non-diabetic group

Parameters	Group I Diabetics (n=22) Mean ± SD	Group II Non-Diabetics (n=22) Mean ± SD	*p*-value
Age (years)	58.86±1.38	58.22±1.85	0.1875
BMI	22.7±1.3	22.1±1.5	0.517
NRF2 (pg/ml)	1.306±0.800	2.309±1.290	0.03*

DISCUSSION

Obesity with higher BMI is the established main risk factor of type II diabetes mellitus. In our study, which included 44 patients of osteoarthritis, age and BMI was found non-significant whereas in a study by Siewert et.al. has reported that diabetic patients had higher BMI when compared to controls.[21] This difference can be because of older age that can lead to muscle loss due to decreased mobility because of OA.[1–3]

Many factors are involved in the pathogenesis of inflammation cenetred conditions like diabetes and osteoarthritis. Hyperglycaemia mediated oxidative stress play's important central role in chronic inflammation thus role of factor modifying oxidative stress are important in pathophysiology of these diseases. Nfk2 acts as the master regulator of intracellular antioxidant pathways will definitely affect oxidative stress inside the cell thus improving inflammation. Nfk2 levels are affected by oxidative stress conditions, inflammation and aging.

Khan *et.al*., in their study found significantly higher expression of Nrf2 and its genes in OA which is similar to results of our study.[24]

In our study, Nrf2 blood expression levels were low in osteoarthritis group I having diabetes compared to osteoarthritis group II that are non-diabetic. Siewert *et.al*. and Sireesh *et.al*., also reported reduced Nrf2 mRNA in whole blood from T2DM compared to controls.[9,21] Jiménez-Osorio *et.al*. also reported that Nrf2 levels were lower in prediabetic and diabetic patients.[22]

Nfk2 expression and binding with DNA are altered in diabetic patients thus affecting the antioxidant activities of cell. Dietary interventions through naturally occurring Nfk2 activating agents in diet may improve antioxidant property of a cell.[23] Oxidative stress in OA accompanying diabetes mellitus negatively affects the levels of protective Nfk2 antioxidant regulator.

CONCLUSION

Oxidative stress is implicated in many diseases including osteoarthritis diabetes mellitus. Nrf2, a transcriptional regulator of many antioxidant genes should be up regulated in response to oxidative stress. Restoring or enhancing Nrf2 levels through dietary modifications in such conditions can be considered as therapeutic potential targets for treatment and prevention of conditions like OA.

AUTHORS' CONTRIBUTION

ZIM, AJ: Concept, literature search. SN, AS: Data collection. SAK, MI: Data analysis, data interpretation.

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