VARIATIONS OF MALONDIALDEHYDE, HYDROXYNONENAL, ISOPROSTANE AND 8-HYDROXYDEOXYGUANOSINE IN SERUM, SALIVA AND URINE IN INDIVIDUALS WITH AND WITHOUT OSTEOARTHRITIS

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Abstract

Background and Objective: Osteoarthritis is a clinical and pathological outcome of a sequence of disorders that ultimately lead to synovial joint structural and functional failure. The aim of this study was to determine the expression of oxidative stress markers like malondialdehyde (MDA), 4- hydroxynonenal (HNE), isoprostane, and 8-hydroxyguanosine in body secretions and mediums like serum, saliva, and urine between patients of OA and non-diseases group.

Methods: The calculated sample size is 50. The age of the participants is ranged from 30-39,40-49,50-59, and 60-69 with a mean score of 54.5+/-9.3 years. Our study design is crossed sectional and comparative. In this study, oxidative stress markers in the body fluids of osteoarthritic patients were compared with the control group of 50healthy individuals in same age group. The levels of stress markers were determined by commercially available quantitative ELISA kits.

Results: Our findings indicate that the highest levels of these oxidative stress markers were detected in serum, followed by urine. The saliva overall presented lower levels of oxidative stress markers in individuals with and without OA. In addition to this, the detection of MDA in serum samples was found to be the most important tool in the detection of OA.

Conclusion: The current study provides an understanding to detect and diagnose the inflammatory markers in the patient's secretions and to treat OA before the disease has pronounced progress. It also helps clinicians in decision making to choose most appropriate test for the early detection of OA.

Keywords: Osteoarthritis, MDA, HNE, 8-hydroxyguanosine, isoprostane, lipid peroxidation.

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Osteoarthritis (OA) is a clinical and pathological disease of articular cartilage that ultimately leads to the structural and functional deformity of the synovial joint.[1] In the beginning, the OA usually involves the entire joint organ, including the subchondral bone, menisci, ligaments, peri-articular muscle, capsule, and synovium.[2] OA is considered the final stage of joint failure, whose initial stage is triggered by some injury to cartilage and ligaments.[3] This disease is more prevalent in women of the age of nearly 60 or older[(4)], causing stiffness and rigidity in joints, tendons, and ligaments, leading to OA. The factors resulting in the development of OA include age, obesity, injury, joint overuse, and some hormonal disorders.[5,6] Chondrocytes are the primary source of reactive oxygen

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species that cause damage to cartilage collagen and affect synovial fluid's viscosity.[7] Some studies have shown that radiographs are insensitive to early disease progression. Bone marrow lesions and the existence of synovitis are not completely imaged on radiographs.[8] Laboratory tests on blood, urine, and synovial fluid are not frequently conducted for diagnosis but could give promising results as such tests enable the early detection of biomarkers.[9,10]

Reactive oxygen species (ROS) are a group of reactive molecules derived from oxygen species and are well recognized for their beneficial and harmful roles.[11] An appropriate balance is required between ROS generation and its clearance.[12] Our focus in this study is to detect oxidative stress markers in patients suffering from OA. Major markers include 8-hydroxy-2-deoxyguanosine, a marker of oxidative DNA damage; malondialdehyde, a marker of oxidation of lipids; hydroxynonenal, another lipid oxidation marker; as well as isoprostane. 8-hydroxy-2-deoxyguanosine is produced as a result of DNA oxidation and serves as a biomarker of oxidative stress.[13,14] As a result of oxidative injury to DNA, guanine gets oxidized, causing guanine and adenine to yield an oxidation product of 8 hydroxyl deoxyguanosine.[15] It is a promising biomarker for the risk assessment of various degenerative diseases; hence, it proves that it increases in the serum and saliva of patients with degenerative oxidative disease. Hydroxynonenal (HNE) is an alpha- and beta-unsaturated hydroxyalkene and acts as a second messenger of oxidative stress.[16,17] Hydroxynonenal reacts with large molecules, such as proteins, and forms protein adducts that are regarded as biomarkers of chronic inflammation.[18] There is no effective treatment for osteoarthritis. Therefore, in biological and physical domains, various studies have been conducted so far for the early detection of biomarkers prevailing in OA, for which this study is also aiming, but within the context of the Pakistani population.

METHODS

The present study was designed as a comparative cross-sectional study with non-probability convenient sampling[19] to recruit study participants. A sample size of 50 was calculated (Epitools® software) for this study using the hypothesized difference of 3.24, with a standard deviation of 1.62 and a variance of 2.64. These values are taken from the reference article.[20]

Fifty (n=50) osteoarthritic female patients in the age group of 50–75 years presented at Sheikh Zayed Hospital, Lahore, Pakistan, were eligible for inclusion in the study. The patients selected had a history of OA not more than three months. Fifty clinically healthy individuals in the same age group were included as controls. Subjects with a history of taking drugs (including alcohol and cigarettes) and pre-diagnosis medications (e.g., antiparkinsonian or antipsychotic) were excluded from this study. It was made sure that none of the controls were on any medication for the treatment of OA or had a history of chronic infections, malnutrition syndrome, depression, psychosis, or metabolic dysfunction (such as diabetes mellitus, liver diseases, or cancer) that could interfere with the levels of oxidative metabolites. Informed consent was obtained from the participants before they were included in this study.

Detection of oxidative stress biomarkers

Serum, saliva, and urine samples were collected from the study subjects (patients and controls) as per Centre for Disease Control and Prevention (CDC) guidelines (CDC, 2018). The levels of all four oxidative stress biomarkers (8-isoprostane, MDA, 4-HNE, and 8-OHdG) in body secretions were qualitatively assessed by the commercially available Abcam competitive ELISA kits (ab201734 for 8-OHdG, ab175819 for 8-isoprostane, ab238537 for MDA, and ab287803 for 4- HNE). A percent positivity of 80% and above was considered positive for the sample.

Descriptive statistics of data compromises of minimum, mean, maximum and standard deviation. For inferential statistics independent sample t-test was applied to compare the levels of each biomarker in the serum between case and control and Tuckey's test was used as post hoc test. Significance was set at $p<0.05$.

RESULTS

The levels of oxidative stress markers, including MDA, IsoP, 8-OHdG, and 4-HNE, were measured in the serum, saliva, and urine of the patients suffering from OA. The level of the above-mentioned markers was also found in the healthy control group (with no OA). Out of the calculated sample size of 50 patients and controls, 40 patients consented to participate in the study with an 80% response rate. The rest of the 10 patients and 10 controls refused to participate in the study.

Levels of malondialdehyde (MDA) in patients with OA and controls

Among the tested samples, the serum samples of the study subjects showed the highest levels of MDA with a mean of 1.86±0.06 nmol/mL ($p<0.03$), followed by the level of MDA in urine with a mean of 0.46± 0.09 nmol/mL ($p<0.05$) (Figure 1). Out of all study subjects, the lowest level of MDA was detected in saliva (0.06 ±0.32 nmol/mL; $p>0.10$). Overall, a significant difference was found between the MDA levels in the urine and serum of subjects and controls. For the healthy controls, the highest levels of MDA were estimated in serum samples (0.96±0.05) of OA patients, followed by saliva (0.05±0.02) and urine samples (0.03±0.01).

Levels of Isoprostane (pg/mL) in patients with OA and controls:

In the current study, a significant difference was found between the isoprostane levels in the serum and urine of subjects and controls ($p \leq 0.05$). Among the tested samples, the serum samples of the study subjects (Figure 2) showed the highest levels of isoprostane with a mean of 150±58.9 pg/mL ($p<0.001$), followed by the level of isoprostane in urine with a mean of 2.16 ±0.45 pg/mL ($p<0.05$). Out of all study subjects, the lowest level of isoprostane was detected in saliva (2.13 ± 0.25 pg/mL; $p>0.05$). For the healthy controls, the highest levels of isoprostane were estimated in serum samples (1.85±0.09) of OA patients, followed by saliva (1.07±0.23) and urine samples (0.90±0.45).

Table 1: Mean levels of MDA in the patients suffering from OA and healthy controls

Groups	Parameters	Minimum	Mean	Maximum	Standard deviation
Control	Serum	0.87	0.96	1.08	0.05
	Saliva	0.017	0.05	0.08	0.02
	Urine	0.01	0.03	0.05	0.01
Subjects	Serum	1.78	1.86	1.97	0.06
	Saliva	0.05	0.32	0.97	0.32
	Urine	0.27	0.46	0.63	0.09

Levels of 8-hydroxyguanosine (nmol/day) in patients with OA and controls:

A significant difference was found between the 8-OHdG levels in the serum and urine of subjects and controls ($p \leq 0.05$). Among the tested samples, the urine samples of the study subjects showed the highest levels of 8-OHdG with a mean of 1.76±0.15 nmol/day ($p< 0.01$), followed by the level of 8-OHdG in serum with a mean of 1.68±0.22 nmol/day ($p<0.05$) (Figure 3). Out of all study subjects, the lowest level of 8-OHdG was detected in saliva (0.52±0.10 nmol/day, $p > 0.05$). For the healthy controls, the highest levels of 8-OHdG were estimated in serum samples (0.57±0.15) of OA patients, followed by saliva (0.51±0.20) and urine samples (0.06±0.01 nmol/day).

Table 2: Mean levels of ISO in the patients suffering from OA and healthy controls

Groups	Parameters	Minimum	Mean	Maximum	Standard deviation
Control	Serum	1.67	1.85	1.99	0.09
	Saliva	0.69	1.07	1.54	0.23
	Urine	0.35	0.90	1.94	0.45
Subjects	Serum	31.8	150	221.7	58.9
	Saliva	1.68	2.13	2.52	0.25
	Urine	1.64	2.16	2.93	0.45

Levels of 4-Hydroxynonenol (4-HNE) (pg/mL) in patients with OA and controls:

Among the tested samples, a significant difference was found between the 4-HNE levels in the serum and urine of subjects and controls ($p \leq 0.05$). The serum samples of the study subjects showed the highest levels of 4-HNE with a mean of 5.98±1.16 pg/mL ($p< 0.001$),

Table 3: Mean levels of 8-OhdG in the patients suffering from OA and healthy controls

Groups	Parameters	Minimum	Mean	Maximum	Standard deviation
Control	Serum	0.37	0.57	0.85	0.15
	Saliva	0.18	0.50	0.77	0.20
	Urine	0.04	0.06	0.09	0.01
Study	Serum	1.25	1.68	1.99	0.22
	Saliva	0.37	0.52	0.68	0.10
	Urine	1.44	1.76	1.98	0.15

followed by the level of 4-HNE in urine with a mean of 4.02±0.39 pg/mL ($p<0.01$) (Figure 4). Out of all study subjects, the lowest level of 4-HNE was detected in saliva (0.84±0.06 pg/mL, $p>0.05$). For the healthy controls, the highest levels of 4-HNE were estimated in serum samples (1.79±0.09 pg/mL) of OA patients, followed by urine (0.64±0.25 pg/mL) and saliva samples (0.32±0.09 pg/mL).

DISCUSSION

Osteoarthritis is a highly prevalent disease that occurs in almost 22% to 39% of individuals, especially in the elderly, and it is the leading cause of disability, suffering, and morbidity (Marks, 2014). Studies have shown the prevalence of knee osteoarthritis (KOA)

Table 4: Mean levels of HNE (pg/mL) in the patients suffering from OA and healthy controls

Groups	Parameters	Minimum	Mean	Maximum	Standard deviation
Control	Serum	1.66	1.79	1.98	0.09
	Saliva	0.18	0.32	0.51	0.09
	Urine	0.21	0.64	1.01	0.25
Study	Serum	3.52	5.97	8.06	1.16
	Saliva	0.70	0.84	0.94	0.06
	Urine	3.35	4.04	4.68	0.39

to be 7.50%, 10.9%, and 13.6% in China.[21] In India and Bangladesh, it is reported to be 5.78% and 10.20%, respectively.[22] A study in Pakistan has shown that 28.00% of the urban and 25.00% of the rural population have knee osteoarthritis (KOA). A study conducted in China found that risk factors like geography, age, BMI, and sex of the patients are highly associated with the occurrence of OA; hence, these factors should be considered while conducting studies on OA[23] In our study, age and sex were closely considered for the inclusion of the patients. Such factors have also been reported by other studies conducted in Germany and India, showing that obesity (42.24%) and menopause (66.7%) are major risk factors associated with the high occurrence of OA.[24]

Many oxidative stress markers found in the body are lipid hydroperoxide, 4-hydroxynonenal, isoprostane, 8-hydroxydeoxyguanosine, malondialdehyde, and allantois. Since malondialdehyde and isoprostane were discovered, studies have shown that they are reliable oxidative damage markers susceptible to quantitative determinants in biological fluids. Studies have shown that osteoarthritis is due to an increased level of oxidative stress and serum malondialdehyde. Studies conducted showed that plasma and serum malondialdehyde levels are significantly higher ($P<.005$) than in healthy subjects at 0.5 mmol/L.[25] So, it is confirmed that the subject experiences oxidative stress, which results in the oxidation of lipids that increase plasma malondialdehyde. Lipid peroxidation generates decomposing end products such as malondialdehyde, hydroxynonenal, and isoprostane. They are measured in plasma and urine as an indirect index of oxidative stress. It acts as a second cytokine messenger.

Our findings indicate that the highest levels of these oxidative stress markers were detected in serum, followed by urine. The saliva overall presented lower levels of oxidative stress markers in individuals with and without OA. In our study, the levels of inflammatory and oxidative stress markers were significantly higher in the serum samples as compared to the urine and saliva. Similar findings have also been found by Amin,[26] where the highest levels of MDA, 8-OHDG, and isoprotane are found in the serum, making it one of the most important samples to detect OA.

The levels of oxidative stress markers were higher (45 nmol/day) in patients with OA as compared to the healthy controls. Our findings are consistent with those of other studies where elevated levels of such markers were evident in OA patients. The higher concentration

of oxidative stress markers is usually correlated with the stage of inflammation, i.e., low in acute OA and higher in chronic OA, which might relate to the higher level of 8-OHDG in patients with chronic OA.[27] Such oxidative stress, leading to increased levels of the markers, may contribute to the pathology of OA. Urine, on the other hand, was found to have a poor expression of these markers, and these findings are in accordance with earlier findings. Moreover, the apparent discrepancy in the level of these markers in urine is usually affected by the time when the sample was collected and the physical activity of that day. Such confounders should be involved in future studies, nonetheless, these factors are found to be non-damaging for the immune system.[27] In addition to this, plasma and serum samples are described as the most useful samples for the detection of various grades of OA (low and high-grade OA).

The detection of MDA alone is considered a gold standard for the detection of OA and has been most frequently used so far, but it has been criticized as having many pitfalls. Our findings are in opposition of this. Still, MDA measurement has strong clinical relevance due to its role in the induction of pro-inflammatory cells. Studies have shown that all patients with primary osteoarthritis have significantly shown an increase in malondialdehyde concentration, hydroxynonenal, and isoprostane in synovial fluid and a decrease in synovial fluid viscosity when compared to control groups. Lipid peroxidation would significantly increase in association with an increase in reactive oxygen species. Grigolo et al conducted a study showing that the malondialdehyde concentration is significantly higher in the case of lipid peroxidation in osteoarthritis.[28] In some studies, the concentration of lipid peroxidation in synovial fluid is low, and it protects them from oxidative damage.[28]

Serum in synovial fluid in osteoarthritis has a significantly high level of oxidative stress markers like malondialdehyde and hydroxynonenal, which are well-characterized end products derived from the peroxidation of cell membrane PUFA. This damaging effect is a chain reaction that provides a continuous supply of free radicals that leads to further peroxidation. Oxidative decomposition of PUFA and membrane lipids leads to a complex mixture of malondialdehyde and hydroxynonenal. So, it is confirmed in this study that increased lipid peroxidation like malondialdehyde and hydroxynonenal in synovial cells of osteoarthritic patients.[29] It has been proven through studies that malondialdehyde, a toxic aldehyde end product of lipid peroxidation, mediates the oxidation of cartilage and results in a cytopathological effect.[30] All the studies in the past have shown that malondialdehyde levels could be high in the synovial fluid of osteoarthritis patients. Reactive oxygen species contribute to cartilage degradation in osteoarthritis through lipid peroxidation, which shows that hydroxynonenal, isoprostane, and malondialdehyde are the three oxidative stress markers that are increasing in the case of osteoarthritis. But malondialdehyde can be used as a severe marker for osteoarthritis disease.[31] This study differs from the previous study in that it informs us that malondialdehyde levels are high in osteoarthritis.

Similarly, the expression of isoprostane in the serum was found to be highest as compared to the other two sample sources.[32] Though isoprostanes are generally stable molecules, their expression in body fluids gives a good estimation of oxidative stress on phospholipids.[32] Isoprostane is found in free form in all biological fluids and in esterified form; it is unaffected by lipid content in the diet. Urinary isoprostane is significantly higher in patients with chronic inflammation and disease, so the presence of isoprostane in plasma and urine may reflect systemic inflammation. Hydroxynonenal is linked to joint pathophysiology, particularly in osteoarthritis, where hydroxynonenal levels are increased in the synovial fluid of osteoarthritis. Wei et al. showed that apoptosis and necrosis osteoarthritis occur by sustaining and amplifying of cell death signaling.[33] Our study also showed elevated expression of 8-OHDG in patients with OA; however, the expression was not as elevated as in the case of MDA. In previous studies, 8-hydroxydeoxyguanosine levels have also been reported to be increased in the serum, saliva, and synovial fluid of patients.[34]

CONCLUSION

The clinical significance of biomarkers of oxidative stress in humans must come from critical analysis of the markers that should give an overall index of redox status in particular conditions. The current study provides an understanding to detect and diagnose the inflammatory markers in the patient's secretions and to treat OA before the disease has pronounced progress. It also helps clinicians in decision making to choose most appropriate test for the early detection of OA.

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