ABSTRACT

Background: Urinary incontinence is a condition that affects a significant proportion of the population. It is a common condition that can affect women and men of all ages with a wide variety of severity and nature. Different risk factors are associated with the development of urinary incontinence. Aim and Objectives: The aim of the present study was to assess the inhibition of oxidative stress marker Superoxide dismutase (SOD) in osteoarthritic and diabetic patients as a risk factor for urinary incontinence. Superoxide dismutase(SOD) is the antioxidant enzyme that catalyzes the dismutation of superoxide anion(O_2^-) into hydrogen peroxide and molecular oxygen. SOD plays an important protective role against cellular and histologic damages that are produced by ROS. It facilitates the conversion of superoxide radicals into hydrogen peroxide, and in the presence of other enzymes it converted into oxygen and water. Materials and methods: The study protocol and all recruitment materials were approved by the ethical board.40 osteoarthritic patients, 40 diabetic patients and 20 controls were taken from OPTM Research Institute, 145 Rashbehari Avenue, Kolkata-700029, India. The oxidative stress marker superoxide dismutase was estimated in 40 arthritic, 40 diabetic and 20 controls. Results: The activity of SOD in control group and group I(osteoarthritic) were 27.86±3.98 and 24.39±6.51. When compared between group I and control group the level of SOD was also significantly decreased in group II than control group and there was statistically significant lower SOD activity in group I as compared with control group (p<0.05). The activity of SOD in control group and group II (diabetic) were 27.86±3.98 and 23.18±3.98. When compared between group II and control group the level of SOD was also significantly decreased in group II than control group and there was statistically significant lower SOD activity in group II as compared with control group (p<0.001).Conclusion: This study revealed that there was a decreased oxidative stress in osteoarthritic and diabetic patients and superoxide radical is inhibited by superoxide dismutase enzyme, decreases SOD activity in osteoarthritis and diabetes, as a risk factor for urinary incontinence.

KEYWORDS: Urinary incontinence, Risk factors, Oxidative stress, Osteoarthritis, Diabetes, Superoxide dismutase (SOD).

1. INTRODUCTION

Urinary incontinence (UI), involuntary urination is any leakage of urine. It can be a common and distressing problem, which may have profound impact on quality of life. Incontinence occurs because of problems with muscles and nerves that help to hold or release urine. In 1998, urinary incontinence was simply a symptom and in 1998 it was considered a disease by the International Classification of Diseases (ICD/WHO).Urinary incontinence was defined by the International Continence Society, as complaint of any involuntary urine leaking.[1] Urinary incontinence can be caused by a weakening of the pelvic muscles and urethra muscles (the tube that connects the bladder with the outside) or by damaged ligaments. When weakened, the pelvic muscles and urethra cannot contract enough to hold urine when stress is placed on them, such as during a strong cough or sneeze. UI can also occur when a person cannot control the bladder muscle.[2] The factors such as increasing age, menopause, weakened pelvic muscles, pregnancy/childbirth, certain medicines (e.g diuretics), urinary tract infection, diabetes, stroke, smoking, multiple sclerosis, arthritis, obesity, caffeine intake, fluid intake and neurological injury or disease are associated

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with incontinence.\textsuperscript{(3)} Urinary incontinence occurs with increasing age because pelvic muscles and urethra muscles are weakening with increasing age.\textsuperscript{(4-5)}

2. Risk factors of Urinary Incontinence (UI)
A risk factor is something that increases our chances of developing a condition or disease. These risk factors are linked to urinary incontinence.\textsuperscript{(3)}

Arthritis such as Rheumatoid arthritis (RA), reactive arthritis (ReA) and osteoarthritis are a group of joint diseases which differ in pathogenesis intensity and rapidity.\textsuperscript{(6)} Osteoarthritis is a chronic degradation of articular cartilage with a possible secondary inflammatory process.\textsuperscript{(10)} Diabetes is higher risk for asymptomatic bacteriuria. The longer a people has diabetes, The higher risk for UTI complications and fungal related UTI, risk of urinary incontinence.

2. A Oxidative Stress and Osteoarthritis
Osteoarthritis (OA, also known as degenerative arthritis, degenerative joint disease), is a clinical syndrome in which low grade inflammation results in pain in the joints, caused by abnormal wearing of the cartilage that covers and acts as a cushion inside joints and destruction or decrease of synovial fluid that lubricates those joints. As the bone surfaces become less well protected by cartilage, the patient experiences pain upon weight bearing, including walking and standing. Due to decreased movement because of the pain regional muscles may atrophy, and ligaments may become more lax.\textsuperscript{(7)} Osteoarthritis is the most common form of arthritis.\textsuperscript{(7)}

2. a. 1. Superoxide dismutase and Osteoarthritis
Human body produce oxygen free radicals and other reactive oxygen species as by products through numerous physiological and biochemical processes. Oxygen related free radical superoxide is produced in the body, primarily as a result of aerobic metabolism.\textsuperscript{(8)} Superoxide dismutase(SOD) is an endogenous antioxidant enzyme that catalyzes the breakdown of the superoxide anion into molecular oxygen (O\textsubscript{2}) and hydrogen peroxide(H\textsubscript{2}O\textsubscript{2}). Copper Zinc and manganese are key components of the two major superoxide dismutase enzymes which have been shown to fight against the reactive intermediaries that are linked to the joint damage in arthritis.\textsuperscript{(10)} Oxygen free radicals are lipid peroxidation inducing agents that cause the depletion of unsaturated fatty acids of the cell membrane, thus inducing loss of cell integrity and functional alteration of cell receptors and enzymes.\textsuperscript{(9)}

2. b. Oxidative Stress and Diabetes
Cardiovascular complications, characterized by endothelial dysfunction and accelerated atherosclerosis, are the leading cause of morbidity and mortality associated with diabetes.\textsuperscript{(11)} There is growing evidence that excess generation of highly reactive free radicals, largely due to hyperglycemia causes oxidative stress, which further exacerbates the development of diabetes complications. Overproduction or insufficient removal of these free radicals result in vascular dysfunction, damage to cellular proteins, membrane lipids and nucleic acids. Stabilizing glucose levels near normal levels is of utmost important.\textsuperscript{(12)}

2. b.1. Superoxide dismutase and Diabetes
Diabetes mellitus is a group of metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both. Oxidative stress and oxidative damage to the tissue are common end points of some chronic diseases like diabetes.\textsuperscript{(13)} Superoxide dismutase(SOD) is the antioxidant enzyme that catalyses the dismutation of superoxide anion(O\textsubscript{2}-) into hydrogen peroxide and molecular oxygen.\textsuperscript{(14)} SOD plays important protective roles against cellular and histological damages that are produced by ROS. It facilitates the conversion of superoxide radicals into hydrogen peroxide, and in the presence of other enzymes it converted into oxygen and water.\textsuperscript{(15)} Overexpression of SOD or the supplements of antioxidants including SOD mimetics, targeted to overcome oxidative stress, reduce ROS, and increase antioxidant enzymes, has been shown to prevent diabetes mellitus.\textsuperscript{(16)}

3. MATERIALS AND METHODS
3. A. Patient study
The study protocol, consent form and all recruitment materials were approved by the ethical board. As arthritis and diabetes are risk factor for urinary incontinence so 40 osteoarthritic patients, 40 diabetes patients and 20 controls were taken from OPTM research Institute,145 Rasbehari Avenue, Kolkata-700029,India.

3. B. Blood Sample collection
5 ml of venous blood samples (with EDTA vial) were collected from osteoarthritic and diabetic patients.Then blood samples were centrifuged at 1000 \times g for 10 min at 4°C. Serum aliquots were obtained after centrifuging of 5ml blood and stored at \textasciitilde -80°C/\textasciitilde -20°C until analyses were carried out.

3. C. Measurement of oxidative stress marker
Superoxide Dismutase (SOD) activity was determined with an ELISA reader at 450 nm by using abcam\textsuperscript{®} kit.

4. Statistical Analysis
Results were expressed as mean and standard deviation (SD). Statistical analysis was carried out by using software (Microsoft office Excel 2016, add-in statistical tool pack). Comparisons between each two variables were conducted by using the student t-test. P values<0.05 were considered to be of statistical significant and P values>0.05 were statistical not significant.

5. RESULTS
A total 80 osteoarthritic and diabetic patients as a risk factors for urinary incontinence were admitted to OPTM Research Institute from 21 July 2015 to 22 December
Among these patients, 40 patients were confirmed to osteoarthritic and 40 patients were confirmed to diabetic. 40 osteoarthritic patients (group I), their ages ranged from 30 to 70 years (Mean age 46.82±13.01) and 40 diabetic patients (group II) their ages ranged from 30 to 62 years (Mean age 44.87±8.88). Table 1. depicts the activity of SOD in control group and group I (osteoarthritic) were 27.86±3.98 and 24.39±6.51. When compared between group I and control group, the level of SOD was significantly decreased in group I than control group and there was statistically significant lower SOD activity in group I as compared with control group (p<0.05).

Table 1: SOD activity in relation to osteoarthritic patients as a risk factor for urinary incontinence [n=no of total samples mean±standard deviation]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group I (Osteoarthritic)(n=40)</th>
<th>Control group(n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>46.82±13.01</td>
<td>41.2±51.74</td>
</tr>
<tr>
<td>SOD activity(U/ml)</td>
<td>24.39±6.51</td>
<td>27.86±3.98</td>
</tr>
</tbody>
</table>

Values represent the mean±standard deviation.
*Statistically significant, compares group I(Osteoarthritic) and control group [p<0.05].

Table 2: SOD activity in relation to diabetic patients as a risk factor for urinary incontinence [n=no of total samples mean±standard deviation]

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Group II (diabetic)(n=40)</th>
<th>Control group(n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>44.87±8.88</td>
<td>41.2±51.74</td>
</tr>
<tr>
<td>SOD activity(U/ml)</td>
<td>23.18±2.92</td>
<td>27.86±3.98</td>
</tr>
</tbody>
</table>

Values represent the mean±standard deviation.
*Statistically significant, compares group II (osteoarthritic) and control group [p<0.001].

Activity of SOD in Serum.
SOD Activity(U/ml).

6. DISCUSSION
Urinary incontinence is a common condition that can effect women and men of all ages. Incontinence occurs because of problems with muscles and nerves that help to hold or release urine. The body stores urine water and wastes removed by kidneys in the bladder. The bladder connects to the urethra, the tube through which urine leaves the body. Bladder smooth muscle (the detrusor) contracts via parasympathetic nerves from spinal cord levels S2 to S4. Urethral sphincter mechanisms include proximal urethral smooth muscle (which contracts with sympathetic stimulation from spinal levels T11 to L2), distal urethral striated muscle (which contracts via cholinergic somatic stimulation from cord levels S2 to S4) and musculo-fascial urethral supports. Incontinence will occur if our bladder muscles suddenly contract or the sphincter muscles are not strong enough to hold back urine. It occurs when muscles are damaged, causing a change in the position of the bladder. Results showed that the oxidative stress marker enzyme superoxide...
dismutase was lower in arthritic and diabetic patients, as a risk factor for urinary incontinence than control. Oxidative stress from oxidative metabolism causes base damage, as well as strand breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species (ROS) generated superoxide radicals. Superoxide free radical (O$_2^-$) is formed as by product in the red blood cells by the auto-oxidation of hemoglobin to methemoglobin. Superoxide spontaneously dismutates to form H$_2$O$_2$ and O$_2$. The reaction is speeded up tremendously by the action of the enzyme dismutase. Superoxide dismutase removes the toxic superoxide radical (O$_2^-$) formed by the partial reduction of oxygen in tissues. Increased levels of superoxide is not detoxified in patients with osteoarthritis due to decreased efficiency of antioxidant enzymatic and non enzymatic mechanisms and may act as mediators of tissue damage. Table 2 showed that there was a lower SOD activity in diabetic patient than control. Because during diabetes, persistent hyperglycemia causes increased production of free radicals, especially reactive oxygen species (ROS), for all tissues from glucose auto oxidation and protein glycosylation. The increase level of ROS in diabetes could be due to their increased production by enzymic superoxide dismutase antioxidants. The level of this antioxidant enzymes influences the susceptibility of various tissues to oxidative stress and is associated with development of complications in diabetes. Diabetes is a common risk factor for urinary incontinence. Investigations within patients with diabetes suggest that microvascular complications increase both the prevalence and incidence of urinary incontinence, physiological, microvascular and neurological complications of diabetes result in changes that impair the function of incontinence mechanisms, including damage to the innervation of the bladder, altered detrusor muscle function or urothelial dysfunction. Table 1 showed that the SOD activity was lower in osteoarthritis than control. This is due to decreasing of erythrocyte SOD in the patients with osteoarthritis represents a evidence of inflammatory syndrome. More evidence become available suggesting oxygen free radical such as superoxide anion and single oxygen (O$_2^-$), are involved in the pathogenesis of osteoarthritis. Oxygen free radicals destroy lipids by a process called lipid peroxidation. In osteoarthritis patients, SOD, an enzyme destroying O$_2^-$, gives systemically or locally, induces a decrease of inflammation. In Figure 1 compared the SOD activity with control and two groups, arthritic and diabetes. SOD activity was lower in arthritic and diabetes patients than control. There was statistically significant lower SOD activity in osteoarthritis patients as compared with control (p<0.05). SOD activity was also lower in diabetic patients than control and also statistically significant lower SOD activity in diabetic patients as compared with control (p<0.001). The majority of osteoarthritis patients are middle aged to elderly aged. Urgency and frequency are common in this group, but when mobility and dexterity are impaired, they can progress very quickly to incontinence. The urethral sphincter is composed of smooth muscle and connective tissue. Urethral smooth muscle plays an important role in incontinence and there is evidence to show that the elastic element of the connective tissue may aid in keeping the urethra closed. Anatomical urinary stress incontinence occurs when there is incomplete urethral sphincter resistance with a corresponding rise in intra-abdominal pressure. This rise in pressure is a normal occurrence, but if the pelvic muscular and surrounding connective tissue become weak or damaged, the bladder neck and proximal urethra are not maintained in the normal position and when pressure of the bladder increases, leakage become a forced action.

CONCLUSION

We can conclude that osteoarthritis and diabetes are common problem of urinary incontinence. Urinary incontinence is a prevalent and important condition that affects the lives of many peoples. Increased awareness of this condition and its affects is required at the public health care provider. Overproduction of free radicals by inflammatory processes in osteoarthritis causes oxidative damage and in diabetes lower SOD level persist hyperglycemia, causes increasing production of free radicals. This influences oxidative stress in various tissue and is associated with development of complications in diabetes. During the disease state increased production of superoxide radical is inhibited by superoxide dismutase enzyme, decreases SOD activity in disease. So SOD is a good marker for assessment of oxidative stress in osteoarthritis and diabetics, as a risk factor for urinary incontinence and community services should be aware for provisional of convenient toilet facilities for patients.

REFERENCE