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ORIGINAL RESEARCH ARTICLE

ASSESSMENT OF GLUTATHIONE REDUCTASE(GR) AND GLUTATHIONE PEROXIDASE(GPX) IN ARTHRITIC AND DIABETIC PATIENT RISK OF URINARY INCONTINENCE.

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Abstract

Background: Urinary incontinence,involuntary urination,is any leakage of urine.It can be a common and distressing problem,which may have a profound impact on quality of life.Different risk factors are associated with urinary incontinence. Diabetes and arthritis are the major risk factors of urinary incontinence. Free radicals are produced during inflammation and hyperglycemia state as a result oxidative stress occurs during diabetes and arthritis.

Aim and objectives: The aim of the present study was to evaluate the activity of two oxidative stress marker such as glutathione reductase and glutathione peroxidase in diabetes and arthritis patient risk of UI.

Materials and methods: The study protocol and all recruitment materials were approved by the ethical board and study was carried out 40 diabetic,40 arthritic patients and 20 healthy control,taken from OPTM Research Institute,145 Rashbehari Avenue,Kolkata-700029,India.Then oxidative stress marker enzymes GR and GPx activity were evaluated.

Results: It was observed that GR activity of diabetic and arthritic patients in all age groups showed a higher statistical significant as compaired to healthy controls($p<0.001$,$p<0.01$) and in two separate age groups(<50 years old and >50 years old),showed a higher statistical significant as compaired to healthy controls($p<0.01$,$p<0.001$,$p<0.01$,$p<0.05$).Besides GPx activity was observed in diabetic and arthritic patients in all age groups and there was a higher statistical significant of arthritic patients as compaired to healthy controls($p<0.001$) and statistically not significant of diabetic patients as compaired to healthy controls with all age groups and <50 years old age groups($p=0.0002$,$p=0.0021$) and statistically significant as compaired to healthy controls with >50 years old age group($p<0.001$).

Conclusion: This study revealed that glutathione radical is activated by glutathione enzyme during disease state and GR and GPx are good oxidative stress markers for evaluation in diabetes and arthritis.risk of UI.

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Introduction:-

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. UI has social, physical, psychological and economic implications for the individual as well as society as a whole. Urinary incontinence is also known as 'loss of bladder control' or 'urinary leakage'. UI is when urine leaks out before get to bathroom. Millions of people have this problem, especially as they get older. Some people may loss a few drops of urine when they cough or laugh. Others may feel a sudden urge to urinate and cannot control it. Urine loss can also occur during sexual activity and can cause great emotional distress. Urinary incontinence(UI) is a multifactorial syndrome produced by a combination of genitourinary pathology, age related changes, that impair normal micturition(is the ejection of urine from the urinary bladder through the urethra to the outside of the body) or the functional ability to toilet oneself,orboth.Until 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]. It may be evaluated as a symptom,a signal or a condition.A symptom is the subjective indicator of a disease,a signal is what can be observed by the doctor and a condition is defined by the presence of urodynamic observation(a study through examinations) associated with characteristic symptoms of urinary incontinence and signals and/or by the non-urodynamic evidence of relevant pathologic process.The prevalence of incontinence in canada is about the same as in the united states-about 10% of the population.That means approximately 3.5 million canadians experience some form of incontinence.Oxidative stress influence the risk factors of urinary incontinence.Oxidative stress is defined in general as excess formation and in sufficient removal of highly reactive molecules such as reactive oxygen species(ROS) and reactive nitrogen species(RNS).ROS include free radicals such as superoxide(O_2·-),hydroxyl(·OH),peroxyl(·RO_2),hydroperoxyl(·HRO_2-) as well as non redical species such as hydrogen peroxide(H_2O_2) and hydrochlorous acid(HOCl).Overproduction of free radicals can cause oxidative damage to biomolecules(lipids,proteins,DNA),eventually leading to many chronic disease such as atherosclerosis,cancer,diabetesarthritis,post ischemic perfusion injury,myocardialinfarction,cardiovasculardiseases,chronicinflammation,stroke and septic shock,aging and other degenerative diseases in humans[2]

Influence of risk factors on urinary incontinence:-

Different risk factors are associated with urinary incontinence.These risk factors influence to all ages of men and women.These risk factors are described below[8].

Gender:-

Women have a significantly higher chance of experiencing stress incontinence than men.Certain aspects of a female's life,such as childbirth and menopause make incontinence more likely.A man's risk is higher if he has prostate gland problems.

Increasing age:-

All olderadults are susceptible to incontinence.One in 10 people over age 65,and 3 in 10 over age 80,have some type of bladder control loss.The muscles in the bladder and urethra are weaken during increasing age.This means the bladder can not hold as much liquid as before,raising the risk of involuntary leakage.This does not mean that people will necessarily become incontinent when they are old,it just means the risk is higher.

Obesity:-

Obesity has been involved as a risk factor for urinary incontinence.There are several mechanical and physiologic reasons why an increased body mass index may be associated with,if not causative of urinary incontinence.Evidence suggests that the prevalence of both urge and stress incontinence increases proportionately to a rising BMI[3].Indeed,the increase in intravesical pressure created by a rising BMI may reduce the continence gradient between the urethra and the bladder.In this situation,the magnitude of increased intra-abdominal pressure necessary to force urine through the urethra is reduced because of static pressure within the bladder is higher[4].There is early evidence that a subset of women with elevated BMIs and urge incontinence may have a β_3-adrenergic receptor mutation that simultaneously affects both insulin sensitivity and β_3-mediated detrusor muscle relaxation[5].

Pregnancy/childbirth/weak pelvic muscle:-
Pregnancy and childbirth can increase the later risk for urinary incontinence.The risk is highest with the first child over age 30.Vaginal birth can cause pelvic prolapsed,a condition in which pelvic muscles weaken and the pelvic organs(bladder,uterus) slip into the vaginal canal.Pelvicprolaps and the surgery used to correct it,can cause incontinence.Similarly,evidence is inconclusive as to whether episiotomy prevents urinary incontinence(Episiotomy is a surgical incision that is made during childbirth to the perineum,the muscle between the vagina and the rectum).

High Impact Exercise:-
High impact exercise are susceptible to urinary leakage,particularly with a low foot arch.Shock to the pelvic area is increased as the foot makes with hard surfaces.

Hysterectomy surgery:-
Hysterectomy is associated with a deterioration of bladder function.Pelvic floor dysfunction can be related to age and postmenopausal degenerative changes of pelvic floor supportive tissues and pelvic organ prolapsed.

Menopause:-
After menopause women produce less estrogen,a hormone that helps keep the lining of the bladder and urethra healthy.Deterioration of these tissues can aggravate incontinence.

Prostatectomy and prostate cancer:-
In men,stress incontinence or urge incontinence can be associated with untreated prostate cancer and prostatectomy(removal of all part of the prostate).But often incontinence is a side effect of treatments for prostate cancer.

Obstruction:-
A tumor anywhere along our urinary tract can block the normal flow of urine,leading to overflow incontinence.Urinary stones-hard stone like masses that form in the bladder sometimes cause urine leakage.

Arthritis:-
Arthritis is a joint inflammation that causes pain and stiffness in the joint.Arthritis is the most common risk factor of urinary incontinence and it is not a single disease but it is an informal way of referring to joint pain or joint disease.People of all ages,sexes and races can and do have arthritis,and it is the leading cause of disability in America.

Diabetes:-
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.

Constipation:-
Constipation are also common problems affecting between 2 and 27% in developed countries [6].The rectum is located near the bladder and shares many of the same nerves.Hard,compacted stool in our rectum causes these nerves to be overactive and increase urinary frequency.

Neurological disorders:-
Multiple sclerosis,Parkinson'sdisease,stroke,a brain tumor or a spinal injury can interfere in bladder control,causing urinary incontinence.

Personal addiction:-
Certain drinks such as alcohol,smoking and coffee(caffeine) people who drinks approximately two cups of coffee each day may be more likely to suffer from urinary incontinence because this addiction is stimulating our bladder and increasing our volume of urine,result of overflow incontinence [7].

Influence of free radical, oxidative stress and level of glutathione on diabetic patient risk of urinary incontinence:-
Free radicals are atoms or groups of atoms that contain an unpaired electron in their valence shell. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) are chemically reactive metabolites classified as free radicals

owing to the presence of one unpaired electron in an oxygen atom and a nitrogen atom, respectively[9],[10]. In biological systems, free radicals are produced naturally and are essential in certain cellular and homeostatic functions. Hyperglycemia caused by diabetes mellitus (both type 1 and type 2) is a major cause of oxidative stress.Research has shown that there are numerous pathways by which hyperglycemia can lead to greater free radical production, and these include mitochondrial respiration,glucose autoxidation, activation of the polyol pathway, and the formation of advanced glycation end products. Larger amounts of NADH and FADH are produced in hyperglycemic conditions owing to glucose autoxidation. Hyperglycemia also results in a depletion of NADPH due to the increased rate of sorbitol synthesis via the polyol pathway. This depletion of NADPH impairs the rate of regeneration of reduced glutathione from oxidized glutathione, as NADPH is used as a co-factor in this reaction resulting in impaired cellularantioxidant capacity [11,12]. Antioxidants comprise a type of defense mechanism against free radicals, as they protect cells by behaving like scavengers of free radicals and thus neutralize their damaging oxidative capacity. Antioxidants can be divided into two different classes based upon their mechanism of action. These are the enzyme antioxidants and the non-enzyme antioxidants.Glutathione peroxidase and glutathione reductase are the major enzyme antioxidant which scavenge free radicals by converting them into less reactive molecules which later may be easily metabolized.

Influence of free radical, oxidative stress and level of glutathione on arthritic patient, risk of urinary incontinence:-

Arthritis is basically the result of the destruction of the cartilage in our joint.Cartilage is the cushion of our joints.It is at the end of our bones and the larger joints,like our knees,have extra for added cushioning.Our joints are also lubricated with synovial fluid to allow everything to move smoothly.Cartilage is constantly being worn down and must be rebuilt at the same rate to keep the joint healthy.When the breakdown happens faster than the building,that's when the joint starts to wear out.Excessive free radical damage in the joint is the underlying problem of arthritis.When the fluid from an arthritic joint is analyzed there is a huge concentration of free radicals.Fluid from a normal joint has no free radicals.Generally free radicals from arthritic joint attack the nearest stable molecule by stealing it's electron.The attack molecule then loses its electron and becomes a free radical itself,beginning a chain reaction cascade resulting in damage to living cells.The formation of free radicals,which is secondary to the production of reactive oxygen species, is part of the physiological processes of aerobic metabolism.Cellular metabolism also produces free radicals and these active radicals can be very useful as a defense mechanism controlled by molecular stimuli or signals against damage cased by microorganisms [13].Glutathione is an antioxidant occurs naturally and exists in each cell in the body. Although glutathione occurs naturally, its production declines with age.That deficiency causes diseases that are related to age, including arthritis. Glutathione deficiency adversely affects the nervous system,leading to problems such as poor balance coordination and of course,aethritis.Oxidative stress defined as an imbalance between oxidative processes and reduction equivalents(antioxidants),is involved in the development of degenerative joint diseases.There is substantial body of published research that suggests that arthritic disease are characterized by inflammation and oxidative stress.Oxidative stress produces ROS that play key roles in the development of arthritis.These dangerous chemicals accumulate in the synovial joint,causing extensive structural damage cell death and inflammation.

Materials and Methods:-

Patient study:-

The study protocol, consent form and all recruitment materials were approved by the ethical board. 40 arthritic and 40 diabetic patients and 20 controls were taken from OPTM research Institute,145Rasbehari Avenue, Kolkata-700029,India.

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 × g for 10 min at 4°C. Serum aliquots were obtained after centrifuging of 5ml blood and stored at ─80°C/─20°C until analyses were carried out.

Measurement of glutathione peroxidase and glutathione reductase:-

Glutathione peroxidise and glutathione reductase were oxidative stress marker. GPx was determined with an colorimetric method or microplate reader at 340nm by using abcam® kit and GR was determined with an ELISA reader at 405 nm by using abcam® kit.

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 and P values<0.05 were considered to be of statistical significant and P values>0.05 were statistical not significant.

Results:-

A total of 40 diabetic and 40 arthritic patients were taken from OPTM Research Institute and glutathionereductase(GR) and glutathione peroxidase(GPx) activity levels were assessed.

Table 1:- Assessment of glutathione reductase in diabetic patient risk of urinary incontinence in all age groups [n=no of total samples mean ± standard deviation].

Subjects	GR activity (mU/ml)
Control group [n=20]	1.39 ± 0.02
Experimental group [n=40]	4.13 ± 1.65

Values represent the mean ± standard deviation
Statistically significant when compares experimental group and control group [p<0.001].

Table2:- Assessment of glutathione reductase in arthritic patient risk of urinary incontinence in all age groups [n=no of total samples mean ± standard deviation].

Subjects	GR activity (mU/ml)
Control group [n=20]	1.39 ± 0.02
Experimental group [n=40]	1.91 ± 0.58

Values represent the mean ± standard deviation
Statistically significant when compares experimental group and control group [P<0.01].

Table3:- Assessment of glutathione peroxidase in diabetic patient risk of urinary incontinence in all age groups.[n=no of total samples mean ± standard deviation].

Subjects	GPx activity (mU/ml)
Control group [n=20]	82.75±4.38
Experimental group [n=40]	76.42±6.43

Values represent the mean±standard deviation
Statistically not significant when compares experimental groups and control group [p=0.0002].

Table4:-Assessment of glutathione peroxidase in arthritic patient risk of urinary incontinence in all age groups. [n=no of total samples mean ± standard deviation].

Subjects	GPx activity (mU/ml)
Control group [n=20]	82.75 ± 2.34
Experimental group [n=40]	25.3 ± 6.43

Values represent the mean ± standard deviation
*Statistically significant when compares experimental group and control group [p<0.001}.

A total of 40 diabetic and 40 arthritic patients risk of urinary incontinence were taken from OPTM Research Institute and oxidative stress markers such as glutathione reductase(GR) and glutathione peroxidase(GPx) activity levels were assessed.Table1 and Table2 depicts the assessment of glutathione reductase activity in all age groups, of diabetic and arthritic patients, risk of UI and healthy control groups. When compaired the glutathione activity levels between risk factors of urinary incontinence (diabetes,arthritis) and healthy controls there were higher GR activity in experimental groups than control groups. There were statistically significant higher GR activity in experimental groups as compaired with control groups [(p<0.001,p<0.01)]. Table3 and Table 4 depicts the assessment of glutathione peroxidase (GPx) activity in all age groups of diabetic and arthritic patients risk of UI and healthy control groups. When compaired the glutathione activity levels between risk factors of urinary incontinence and healthy controls there were lower GPx activity in experimental groups than control groups. There was statistically not significant lower GPx activity in experimental groups of diabetes as compaired with control groups

[(p=0.0002)] and statistically significant lower GPx activity in experimental groups of arthritis as compaired with control groups [(p<0.001)].

Discussion:-

Urinary incontinence is a multifactorial syndrome produced by a combination of genitourinary pathology, age related changes and comorbid conditions that impair normal micturition of the functional ability to toilet oneself, or both. Incontinence is a prevalent health condition that is rarely discussed as people living with the condition are often embarrassed to discuss it with their healthcare providers; urinary incontinence is a condition in which involuntary loss of urine by the "International Continence Society Standardization Committee". In addition to the urethra, urine can also leak from an extraurethral source such as fistulas or congenital malformations of the lower urinary tract [14].Urinary incontinence may result from years of combinatory impact of environmental assaults and genetic susceptibility. As a major environmental risk factor is oxidative stress. Oxidative stress not only results in accumulation of reactive oxygen species(ROS) but also damages DNA or modifies DNA structures at an epigenetic level. Biomarkers can be employed to reflect environmental prooxidant exposures and dietary antioxidant intake or to serve as a surrogate measure of a disease process. Biomarkers of oxidative stress have the potential to help establish pathogenic stages of and risk for disease and should be employed to inform the design and outcome measures of clinical trials. A free radical can be defined as any molecular species capable of independent existence that contains an unpaired electron in an atomic orbital. The presence of an unpaired electron results in certain common properties that are shared by most radicals. Many radicals are unstable and highly reactive. They can either donate an electron to or accept an electron from other molecules, therefore behaviouring as oxidants or reductants. Results showed the activity of glutathione reductase and glutathione peroxidase in diabetes and arthritis disease due to the production of glutathione radical during disease state. Glutathione(GSH),a tripeptide, y-L-glutamyl - L cysteinylglycine, is present in all mamalian tissues at 1-10mM concentrations (highest concentration in liver) as the most abundant nonproteinthiole that defends against oxidative stress [15].GSH can maintain SH groups of proteins in a reduced state, participate in amino acid transport, detoxify foreign radicals, act as coenzyme in several enzymatic reactions, and also prevent tissue damage [16].It is an efficient antioxidant present in almost all living cells and is also considered as a biomarker of redox imbalance at cellular level [17]. As a consequence of increased oxidative status, GSH showed the frequent alteration in its concentration. Inflammation and altered antioxidant profiles are the usual complications in diabetes mellitus as results decreased GSH/GSSG ratio [18].Glutathione reductase plays an important role through the reduction of GSSG to GSH and oxidation of NADPH to NAD^+ in diabetes and arthritis state. However GSH can be reclaimed from GSSG through the use of glutathione reductase (GSR) by the use of NADPH as a cofactor. Result showed the activity of glutathione in arthritis, risk of UI. In arthritis there was significant reduction in GPx in patients than healthy control suggesting the role of oxidative stress in arthritis this goes with the result of Surapaneni and Venkataramanna[19] showing that altered antioxidant enzyme activities and lipid peroxidation are considered to be the major phenomenon by which ROS can cause cartilage collagen degradation [19].

Conclusion:-

We can concluded that overproduction of free radical during disease state influence oxidative stress.Oxidative stress is a situation in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them.Oxidative stress lead to connective tissue degradation and joint and periarticular deformities [20,22] in joint related disease such as arthritis,risk of urinary incontinence.Oxidative stress also influence diabetes risk of UI because persistent hyperglycemia causes increased production of free radicals,especially reactive oxygen species,for all tissues from glucose auto oxidation.During the disease state increased production of glutathione radical is activated by glutathione enzyme.So GR and GPx are good markers for evaluation of oxidative stress in risk factors of UI.

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