Uric Acid as a Natural Scavenger of Peroxynitrite in a Sample of Iraqi Patients with Rheumatoid Arthritis

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Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory disease associated with decreased antioxidant state .This study aim to investigate the status of oxidant/antioxidant in a sample of Iraqi patients with RA and the role of peroxynitrite and its natural scavenger uric acid in them .This case-controlled study was conducted at Baghdad teaching hospital /Baghdad from December 2010-May 2011 . Twenty-five patients with mean age 39 years and 25 apparently healthy subject as controls with mean age 29 years were included in the study .Investigations include estimation of serum levels of nitric oxide (NO) ,peroxynitrite (PN) , malondialdehyde (MDA) , and uric acid (UA) .Serum PN levels were significantly elevated in RA patients as compared with control subjects , UA levels were found significantly lowered in RA patients as compared with control subjects. No significant differences were found between NO and MDA among patients and controls group .In conclusion :this study demonstrated decreased serum uric acid levels in patients with RA accompanied by decreased its effect as a natural scavenger of PN.

Key word : Rheumatoid arthritis ,Peroxynitrite ,Uric acid.

دور حامض اليوريك ككاسح طبيعي للبيروكسينترات لدى نموذج من المرضى العراقيين المصابين بالتهاب المفاصل الرثوي

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الخلاصة

التهاب المفاصل الرثوي هو مرض التهابي مزمن مرتبط مع انخفاض معدلات مضادات الاكسدة. تهدف هذه الدراسة لتقييم التوازن بين حالتي الاكسدة و مضادات الاكسدة لدى نموذج من المرضى العراقيين المصابين بهذا المرض وكذلك دور البيروكسينترات و دور الكاسح الطبيعي له وهوحامض اليوريك لدى هؤلاء المرضى .اجريت هذه الدراسة في مستشفى بغداد التعليمي –بغداد- العراق في الفترة من كانون الاول ٢٠١٠ لغاية ايار ٢٠١١ . شارك في هذه الدراسة ٢٥ مريض مصاب بالتهاب المفاصل الرثوي متوسط اعمارهم ٣٩سنة و٢٥ شخص سليم متوسط اعمارهم ٢٩.٩سنة تم اختيارهم كمجموعة السيطرة وتم تقييم معدلات اوكسيد النترات ,البيروكسينترات ,المالوندايالديهايد وحامض اليوريك في نماذج الدم المسحوبة من المجموعتين .اظهرت النتائج ارتفاعا معنويا في معدلات البيروكسينترات لدى مجموعة المرضى بالمقارنة مع مجموعة السيطرة , بينما كانت معدلات حامض اليوريك منخفضة انخفاضا معنويا لدى المرضى بالمقارنة مع مجموعة السيطرة , كان هناك فرقا غير معنوي في معدلات اوكسيد النتيرات والمالوندايالديهايد بين المجموعتين .اشارت الدراسة الى انخفاض معدلات حامض اليوريك لدى هؤلاء المرضى مرتبط مع انخفاض دورحامض اليوريك ككاسح طبيعي لجذر البيروكسينترايت .

الكلمات المفتاحية :التهاب المفاصل الرثوي ، البيروكسينترايت ، حامض اليوريك .

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory and autoimmune disease [(1)], characterized by a chronic hypertrophic synovitis leading to destruction of connective tissues and functional damage of cartilage and bony structure [(2)]. There is evidence indicating that a low antioxidant status is associated with a higher risk of developing RA [(3)]. The rheumatoid inflammation is associated with an increased generation of oxidants (reactive oxygen and reactive nitrogen species) , which play an important role in the inflammatory process and contribute to tissue destruction [(4)].Several mechanisms exist whereby oxygen radicals might be generated within the joint in RA ,these include the release of such species from activated synovial macrophages and polymorphs [(5)],the prostaglandin pathway [(6)] and xanthine oxidase mediated synovial ischemic reperfusion injury [(7)]. Nitric oxide (NO) is a biological messenger mediating many important physiological functions but also pathological process .It play a vital role in host defense and immunity by modulating inflammatory processes [(8)]. The increased production of NO has been linked to both protective and proinflammatory mechanism associated with tissue damage in inflammatory diseases [(9)]. During acute and chronic inflammation ,superoxide is produced at rates that overwhelm the capacity of the endogenous superoxide dismutase (SOD) enzyme defense system to remove it [(10)] ,superoxide interacts with and destroys the biological activity of

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NO [11],the rate of interaction between superoxide and NO is faster than the rate of removal of superoxide by SOD[12] .Peroxynitrite is formed by the rapid combination of NO and superoxide in a reaction that is limited only bydiffusion rate of the molecule [13]. Under normal physiological conditions ,there is a low production of peroxynitrite resulting in a minimal amount of oxidative damage [14] ,while during inflammatory conditions ,large amount of NO and superoxide anion are produced ,leading to the formation of a strong oxidant ,peroxynitrite anion [15]. The proposed cytotoxic properties of peroxynitrite include protein nitration, lipid peroxidation, inhibition of cellular metabolic pathways & signal transduction mechanisms and DNA strand breakages [15].

Important targets for oxidants are the unsaturated fatty acids in cell membranes. Malondialdehyde (MDA) is a product of lipid peroxidation and thereby functions as a marker of oxidative stress [16], earlier studies have shown that the level of MDA is related to RA disease activity [17].Uric acid is a metabolic product of purine metabolism that may function as an antioxidant [18] .Some disease states such as gout have been shown to result when UA levels in the blood are too high ,while other conditions such as neurodegenerative diseases ,may be caused by reduced serum UA levels. The manipulation of serum UA levels has become a popular strategy in the treatment of a variety of disease [19]. Apart from being able to scavenge peroxynitrite, UA also scavenge singlet oxygen, peroxy and hydroxyl radicals, ozon and hypochlorous acid [20]. By using rat zymosan-induced arthritis; the administration of uric acid, in addition to reducing the inflammatory parameters, also prevents the loss of articular cartilage [21] .In this study we aimed to study the role of nitric oxide and peroxynitrite in RA patients and the role of uric acid as a scavenger of these free radical in those patients and if manipulation of serum UA levels is one of the methods in the treatment of RA.

Patients and Methods

This study was conducted at Baghdad Teaching Hospital from December 2010-May 2011. Twenty-five patients with RA; their ages ranged from 17-55 years who were attending the rheumatology consultation clinic of Baghdad Teaching Hospital, and twenty-five apparently healthy subjects as control group with age range of 19-50 years were included in the study after inform consent .RA was diagnosed on the basis of the revised criteria of the American college of Rheumatology (ACR) [22]. Exclusion criteria were pregnancy ,the presence of active infection and the presence of cancer ,since all can affect serum NO level .Ten milliliter blood samples were collected from all patients by vein puncture , 2 ml of each sample were transferred to anticoagulant tube [EDTA (ethylene diamine tetra acetic acid)] tube for erythrocyte sedimentation rate (ESR) determination according to the Westergreen method [23] .The rest of 8ml were transferred to 10 ml sterile plane tube, allowed to clot for 30 minutes at room temperature and centrifuged at 3000 rpm for five minute to obtain serum .Serum aliquots were divided into four 1 ml eppendroffs tubes for nitric oxide, peroxynitrite ,MDA and uric acid estimation .Measurement of NO level was performed according to the method of Miranda et al (2001), absorbance was read at 540nm using ELISA reader (BioTek ELX50 USA) [24]. Serum peroxynitrite level was determined according to the method described by Beckman et al [25], cited by Vanuffelen et al [26], in which the peroxynitrite mediated nitration of phenol was measured spectrophometrically (ECM LAB spectrophotometer ,Germany) at 412 nm. The levels of serum UA was measured spectrophotometrically with kit from Biomaghreb company (France) .Malondialdehyde level was estimated as described by Hunter et al [27] ,the absorbance of the supernatant was determined at 530 nm spectrophotometrically (ECM LAB spectrophotometer ,Germany) .

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 12 (SPSS Inc., Chicago, IL., USA) .Mean and standard error (SE) were calculated. Student t-test was used to evaluate the significance (*P*-value) between study variables .A *P*-value of<0.05 was considered statistically significant.

Results

Table -1 show the demographic characteristics of the subjects .There were non-significance difference between patients group and control subjects regarding gender, age and body mass index. Female represent 40% of total patients ,while male represent 60% . Among control subjects , female represent 48% of total group and male represent 42% . The mean age of patients group was (39.19 years) with age range (17-55) years. The mean age of control subjects was (29.9 years) with age range (19-50) years. The mean BMI of patients group was (25.5kg/m^2) ,with range of (21.3-35.2) kg/m^2.The mean BMI of control

subjects was (24.9kg/m^2) ,with range of (18-36.7) kg/ m^2.

Serum analysis showed significant elevation in ESR levels of patients group 88.33 ±4.38 (mean ±SE) mm/hr as compared with control subjects 14±1.29 (mean ±SE) mm/hr ,(P<0.05) . There was non-significant decrease in serum NO levels in patients group 163.7±23.6 (mean±SE) µmol/l as compared with control subjects 164.3±9.22 (mean ±SE) µmol/L,(P>0.05) ,while there was significant increase in serum levels of peroxynitrite in patients group as compared with control subjects 8.71±1.16 (mean±SE) µmol/l,(0.53±0.04) (mean±SE) µmol/l respectively ,(P <0.05) .There was non-significant increase in serum levels of MDA in RA patients group 4.76 ±0.31 (mean±SE) nmol/ml as compared with control subjects 4.01 ±0.47 (mean±SE) nmol/ml, (P >0.05) .The serum levels of UA were significantly decreased in patients group 4.61 ±0.73 (mean±SE) mg/dl as compared with control subjects 8.3 ±0.64 (mean±SE) mg/dl (P <0.05)

Table-2 shows the levels of erythrocyte sedimentation rate, nitric oxide, peroxynitrite, malondialdehyde and uric acid in patients with rheumatoid arthritis and healthy control subjects .

Table1:Demographic data of studied groups describe in means ±standard error

Parameter	RA patients	Control
Number	25	25
Gender F/M	10/15	12/13
Age (years)	39±2.5	29.9±2.1
BMI(kg/m^2)	25.5±0.62	24.9±0.79

BMI: body mass index .
F/M: female /male .

Table 2: Mean±standard error of nitric oxide, peroxynitrite, malondialdehyde, uric acid and erythrocyte sedimentation rates of patients with rheumatoid arthritis and healthy controls group

Parameter	Patients group	Controls group	P value
NO(µmol/l)	163.7±23.6	164.3±9.22	0.97
$ONOO^-$ (µmol/l)	8.71±1.16	0.53±0.04	0.00*
MDA(nmol/l)	4.76±0.31	4.01±0.47	0.183
ESR(mm/hr)	88.3±4.38	14±1.29	0.00*
Uric acid (mg/dl)	4.61±0.73	8.3±0.64	0.003*

NO: Nitric oxide
$ONOO^-$:Peroxynitrite .
MDA:malondialdehyde
ESR:erythrocyle sedimentation rate

Discussion

Despite the synovial tissue being highly vascularized , the rheumatoid joint is recognized as a site with typical biochemical features of hypoxia-induced oxidative stress [28] , the accumulation of oxidized DNA ,proteins and lipids within the inflamed rheumatoid joint provides evidence for the damaging effects of radicals in this pathology [29] . It was recently reported that NO by itself is protective to chondrocyte under oxidative stress in vivo, while reactive oxygen species, including peroxynitrite, promote chondrocyte death [30]. In one study Taysi et al [31] found that serum MDA correlated positively with disease activity score ,and Deaney et al [32] have reported correlation between ESR and MDA . The result of our study show non-significant increase in serum MDA of RA patients compared to controls and correlate with results of Kajanachumpol et al study that showed no significant change in MDA levels in patients with RA compared to controls [7] . While in another study ; most patients had low to moderate disease activity ,no correlation between urine MDA and disease activity variables were found [33]. The serum levels of NO of both groups were not different despite the expected elevation in serum NO levels in patients group due to the increase of superoxide production which leads to increase in formation of peroxynitrite as it found in this study,the elevation in peroxynitrite level could explained as a result from increasing nitric oxide and super oxide reaction .

Uric acid is a natural antioxidant, accounting for up to 60% of the free radical scavenging activity in human blood [34], serum urate concentration in RA correlated inversely with oxidative changes in serum albumin and immunoglobulin G, it is suggested that serum urate might have an antioxidant role under certain conditions by limiting free radical induced oxidative changes to protein during inflammation [35]. Although chronic gout and rheumatoid arthritis are common clinical entities, they seldom coexist [36]. In one study the plasma level of uric acid were inversely related to indices of RA disease activity [33]. Peroxynitrite ,in particular , is believed to have a significant negative impact on cellular function and survival [14] .UA may assist in the removal of superoxide by preventing against the degradation of superoxide dismutase [14] , removal of superoxide helps to prevent its reaction with nitric oxide , blocking the formation of peroxynitrite [37].UA is also very effective at preventing peroxynitrite from nitrating the tyrosine residues of proteins ,

thereby preventing the inactivation of cellular enzymes and modification of the cytoskeleton [(14)].UA also has the ability to bind iron ,and inhibit iron –dependent ascorbate oxidation ,preventing an increased production of free radicals that can further contribute to oxidative damage [(38)] ,thus a reduced UA concentration may decrease the ability of the body to prevent peroxynitrite and other free radicals from acting on cellular components and damaging the cell [(39)] .The decrease in UA levels may be attributed to its oxidation by peroxynitrite and formation of allantoin , described to occur in humans [(40)] .Patients in this study showed a significant decrease in serum levels of uric acid when compared to control subjects , in turn this can result in decrease protective effect of uric acid as a natural scavenger of peroxynitrite which will lead to further damage in those patients. So these patients are unable to prevent free radical toxicity, leading to the development of inflammation and destruction of tissues . On the other hand, the inflammation that occur in RA leads to the consumption of UA to scavenge the excess free radicals produced ,resulting in a lower UA level .We propose for further studies to estimate the relation between UA and oxidation markers other than PN such as glutathione and also study the role of other antioxidant in RA disease process. In conclusion, we can conclude that patients with RA showed lowered serum uric acid levels accompanied by decreased its activity as natural scavenger of peroxynitrite , while the levels MDA and NO between both groups showed non-significant difference .

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