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Comparison of plasma antioxidant levels in middle-aged and old male with idiopatic osteoporosis: preliminary data

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Abstract. – OBJECTIVES: With the purpose of evaluating the role of oxidative stress (OS) in male idiopatic osteoporosis, we have evaluated plasma total antioxidant capacity (TAC) in patients classified according to age (< 65 or ≥ 65 yrs), with normal hormone values and in age-matched healthy control subjects.

PATIENTS AND METHODS: TAC was evaluated with a colorimetric method, using the system metamyoglobin-H2O2 and the chromogen ABTS; the latency time (LAG, sec) in the appearance of ABTS radical species is proportional to antioxidant content of the system.

RESULTS: We found slightly increased LAG values in middle-aged patients, compared with age-matched controls, probably expression of a compensatory mechanism to OS; on the contrary aged patients showed significantly lower LAG values in comparison with age-matched controls, suggesting a defective compensatory mechanism and, therefore, a risk for oxidative damage.

CONCLUSIONS: OS could be a possible mechanism underlying male osteoporosis, both in middle-aged and aged patients, but compensatory mechanisms seem to be defective in the last group.

Key Words:
Oxidative Stress, Antioxidants, Osteoporosis, Ageing.

Introduction

Osteoporosis is a disease involving an increasing number of patients due to the aging of the population, as reported in epidemiologic surveys[1-5]. Typically considered a female matter particularly connected with a specific period of life (menopause), osteoporosis is in reality very common also in men, considering that they suffer one third of all the hip fractures[6] and that they have a higher first-year mortality[7]. Surprisingly a half of all vertebral fractures happens with values of BMD (bone mineral density) above the osteoporotic standard criteria[8].

Because of these data, the attention and the studies about this problem are growing. The occurence of fractures is surely connected to the age, even if around 50% of these fractures is suffered before 80 yrs[9]. In particular, 34.7% of males between 70 and 85 yrs satisfied the criteria for osteoporosis and 47% of male older than 50 yrs can be considered osteopenic[10].

There are many different factors influencing the bone and among them the hormones play an important role. In particular, the insulin like growth factor (IGF) is important to maintain the trabecular bone, whilst testosterone and estrogens are important for the cortical bone. The physiological decline of these hormones with the aging brings to the higher bone fragility in old people[11,12]. Men generally reach the critical level of fragility 10 yrs later than women because of the higher peak of Bone Mineral Density (BMD) in youth and for the absence of a sudden deprivation of estrogens caused by the menopause[13].

Among the possible causes of idiopathic osteoporosis there is the oxidative stress (OS), caused by the unbalancing between production of free radicals, molecules characterized by high reactivity due to one or more unpaired electrons in the external orbital, and antioxidant defenses in the biological systems[14]. OS has been implicated also as mediator of hormonal influences on bone: radical oxygen species (ROS) greatly influence the generation and survival of osteoclasts, osteoblasts and osteocytes and loss of estrogens and androgens decrease defense against OS in bone[15]. Despite recent studies investigated the

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cellular mechanisms implicated in such a modulation, few data are available in humans.

With the purpose of evaluating the role of OS in human male idiopatic osteoporosis, we have studied plasma total antioxidant capacity (TAC) in patients classified according to age (< 65 or ≥ 65 yrs), with normal hormone values and in age-matched healthy control subjects.

Patients and Methods

Among out-patients consulting our Centre for osteoporosis, we selected 21 patients, affected by idiopathic osteoporosis, confirmed by normal endocrine picture (see below). They presented back pain/spine fracture as a consequence of trivial trauma; they were divided in two groups, according to age: group 1 (n = 13, middle aged patients, range 48-64 yrs) and group 2 (n = 8, older patients, range 65-74 yrs); groups of healthy age-matched subjects (middle age, n = 10, range 30-48 years; ageing controls, n = 5, age 65-78 yrs) were also evaluated.

Criteria of exclusion were: diabetes mellitus, liver or kidney chronic failure, corticosteroid therapy, hyperparathyroidism, obesity, malabsorption or other gastroenteric diseases, neurological diseases.

An endocrine evaluation including testosterone (T), dihydrotestosterone (DHT), estradiol (E2), insulin-like growth factor-1 (IGF-1), free triiodothyronine (fT3), free thyroxin (fT4), thyroid-stimulating hormone (TSH), cortisol levels was performed; bone metabolic parameters were also evaluated (PTH, Vitamin D, osteocalcin, beta-cross laps). For the evaluation of antioxidant systems, a blood sample was collected at 08.00, after overnight fast, immediately centrifuged and stored at -80° until assayed. Finally, bone mineral density was assessed by DEXA.

The following methods were used for hormone assay: Radio Immunoassay (RIA) for DHT (normal range 0.30-0.85 ng/ml); Electrochemiluminescent method (ECLIA) for IGF-1 (n.r. 80-330 ng/ml), PTH (n.r. 19-65 pg/ml), Osteocalcin (n.r. 10-45 ng/ml), Beta Crosslaps (n.r. 0.2-0.7 ng/ml); Chemiluminescent Microparticle ImmunoAssay (CMIA) for T (n.r. 0.20-2.00 ng/ml), E2 (normal values <44 ng/ml), TSH (n.r. 0.35-2.80 μUI/ml), fT3 (n.r. 2.3-4.2 ng/ml), fT4 (n.r. 8.5-15.5 pg/ml), Chemo Luminescent Immunoassay for Vitamin D (n.r. 31-100 ng/ml).

Total Antioxidant Capacity (TAC) was evaluated, with a modification of the method developed by Rice-Evans and Miller[16], as previously described[17]. The method is based on the antioxidants inhibition of the absorbance of the radical action 2,2'-azinobis (3-ethylbenzothiazoline-6 sulphonate) ($ABTS^{\cdot+}$) formed by interaction between ABTS (150 μM) and ferrylmyoglobin radical species, generated by activation of metamyoglobin (2.5 μM) with H_2O_2 (75 μM). Aliquots of the frozen plasma were thawed at room temperature and 10 μl of the samples were tested immediately. The manual procedure was used with only minor modifications, i.e., temperature at 37° C to be in more physiological conditions and each sample assayed alone to carefully control timing and temperature. The reaction was started directly in cuvette through H_2O_2 addition after 1 min equilibration of all other reagents (temperature control by a thermocouple probe, model 1408 K thermocouple, Digitron Instrumentation Ltd, Scunthorpe, United Kingdom) and followed for 10 min under continuous stirring, monitoring at 734 nm, typical of the spectroscopically detectable $ABTS^{\cdot+}$. The presence of chain-breaking antioxidants induces a lag time (the Lag phase) in the accumulation of $ABTS^{\cdot+}$ whose duration is proportional to the concentration of this type of antioxidants. Antioxidant capacity afforded by

Table I. Mean ± SEM hormone values in the two groups of patients studied.

	Group 1	Group 2
Testosterone (ng/ml)	4.76 ± 0.32	3.73 ± 1.26
Dihydrotestosterone (ng/ml)	0.36 ± 0.06	0.38 ± 0.17
Estradiol (pg/ml)	26.72 ± 2.98	34.83 ± 3.84
IGF-1 (ng/ml)	174.5 ± 20.75	113.6 ± 7.81
fT3 (pg/ml)	3.24 ± 0.12	3.36 ± 0.20
fT4 (pg/ml)	12.53 ± 0.44	11.78 ± 0.77
TSH (μU/ml)	1.23 ± 0.13	1.58 ± 0.25
Cortisol (ng/ml)	133.8 ± 14.14	114.57 ± 23.43

Table II. Mean ± SEM values of bone metabolism parameters in the two groups of patients studied.

	Group 1	Group 2
PTH (pg/ml)	40.86 ± 5.24	40.52 ± 5.34
Vitamin D (ng/ml)	19.98 ± 1.97	25.05 ± 4.71
Osteocalcin (ng/ml)	16.85 ± 1.70	13.68 ± 1.81
B-crosslaps (ng/ml)	0.38 ± 0.06	0.35 ± 0.07

chain-breaking antioxidants is expressed as length of Lag phase (sec). Trolox, a water-soluble tocopherol analog, was used as a reference standard and assayed in all experiments to control the system. Absorbance was measured with a Hewlett-Packard 8450A UV/Vis spectrophotometer (Palo Alto, CA, USA) equipped with a cuvette stirring apparatus and a constant temperature cell holder. Measurements of pH were made with a PHM84 Research pH meter (Radiometer, Copenhagen, Denmark); the electrode response was corrected for temperature. Unless stated differently, experiments were repeated two to three times; qualitatively similar results were obtained with individual values varying < 8%.

In the Lag mode, the assay mainly measures nonproteic and nonenzymatic antioxidants that are primarily extracellular chainbreaking antioxidants, such as ascorbate, urate and glutathione.

Statistical Analysis

Distribution of data was estimated by the test of Kolmogorov-Smirnov. Since the data were not distributed normally, the comparison among groups was made using Mann-Whitney U test. The Software Arcus Quickstat (Software Publishing Biomedical version 1.2) was used for this statistic analysis.

Results

Table I shows the mean ± SEM values of the hormone studied in the two groups of subjects; they were all in the normal ranges according to the inclusion criteria. The only significant difference between middle aged and older patients was observed in IGF-1 levels, as expected.

Table II shows the mean ± SEM values of bone metabolism parameters.

Figure 1 shows the mean values of TAC, expressed as LAG, in the two groups of patients, in comparison with age-matched controls. In group 1 osteoporotic patients, LAG values showed a trend toward greater levels of controls; group 2 patients, with lower levels than group 1, exhibited an opposite pattern, with a significant decrease in LAG values in comparison with age-matched ageing healthy subjects.

Discussion

Actually osteoporosis is classified in three different types depending on the causes[3]. The first is the "Involutional or senile" osteoporosis. Steroid hormones play a central role in male bone metabolism: estrogens modulate up to 70% of bone resorption and testosterone the left 30%[18]. Furthermore several authors have demonstrated the androgen receptor (AR) importance for remodeling and formation of bone, promoting osteoblasts activity[19]. AR deficien-

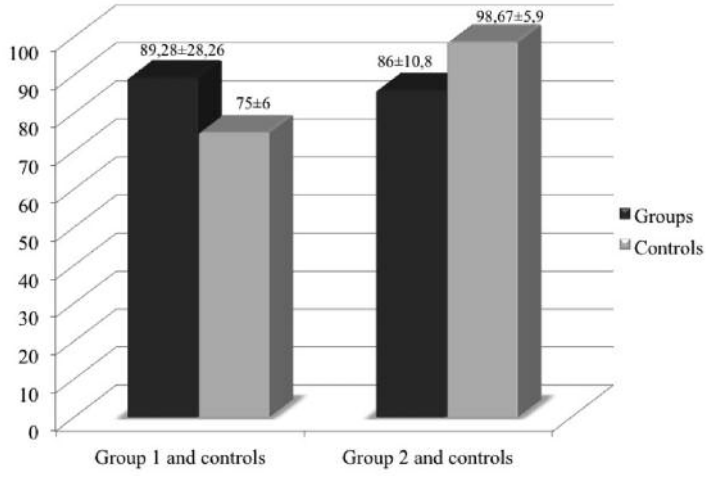

Figure 1. Mean ± SEM values of total antioxidant capacity, expressed as LAG (sec) in the two groups of patients studied.

cy seems also to activate the expression of the receptor activator of NF-kB ligand (RANKL) gene, which causes osteoclastogenesis.

Sex hormone binding globulin (SHBG) increases with the age causing a more relevant descent of bioavailable steroid hormones[20]. This will cause a reduction especially of periosteal bone formation[11,20].

The rise of SHBG in the aging is still unclear, but there is at least one clear correlation with IGF-1, which inhibits its production by the liver and whose levels and inhibition are reduced in old people[21].

Testosterone, Growth Hormone (GH) and IGF-1 act together in the periosteal apposition contrasting the endosteal resorption[20] and their levels are important not only in elderly men to contrast the resorption, but also in young people. If these hormones are low in youth, that causes a lower peak bone mass which will bring an earlier condition of bone fragility and a higher probability of osteoporotic fractures[3].

The second type is "Idiopathic in middle aged males". Almost a half of the cases of male osteoporosis (40%) are classified as primary or idiopathic osteoporosis[12].

Idiopathic osteoporosis is more frequent in younger persons, but can be present in any age group.

Genetic studies have been performed in these patients finding special gene polymorphisms that could cause a lower bone mass, such us Vitamin D receptors; collagen specific proteins (COLIA 1 and COLIA 2); lipoprotein receptor-related protein.

The third type is "Secondary osteoporosis"[20]. In this case a dominat hormonal or metabolic alteration is the main feature causing the bone disorder. Among these, the best known are: *hypogonadism*, for the direct effects on bone and for the collateral effects on muscles which causes less protection against fractures for falls; *Vitamin D deficiency*: This Vitamin determines the absorption of calcium in the intestinal tract. Low levels of it leads to bone loss and chronically to osteoporosis[22]. Daily requirements of Vitamin D are frequently insufficient in some regions[23,24]. This deficiency reduces the quantity of ionized calcium which causes a rise in parathyroid hormone (PTH) levels. This hormone will activate osteoclasts that will "destroy" bone matrix to release the calcium there contained in the bloodstream. This mechanism causes the loss of bone mass and reduces BMD. *Poor calcium intake*: Daily requirement of calcium is 1200 mcgr and in people beyond the 65 is often below this level. The association with Vitamin D deficiency is not uncommon and has a worse effect on bone mass[25]. *Lifestyle*: several wrong habits can have bad effects on bone metabolism and cause osteoporosis. There is a clear correlation with tobacco: smokers have generally a lower bone mass[26]. Also the chronic consumption of alcohol has consequences in metabolism in general and in bone in particular[27,28]. Finally there is not enough evidence that a high coffee consumption would be involved in any mechanism causing osteoporosis as previously assumed[29].

Other causes include (Herrera et al[3]): hormonal therapy (such as androgen deprivation therapy in patients with prostate cancer, anticonvulsant therapy, prolonged steroid therapies, rheumatoid arthritis or ankylosing spondylitis, primary hyperparathyroidism, hepatic or renal disease, malabsorption syndromes, transplanted patients or those treated with immunomodulators, thyrotoxicosis, diabetes mellitus, hypercalciuria, human immunodeficiency virus (HIV).

Except for some specific disease, many of these factors can be coexistent in ageing people, making difficult to establish a definite etiology.

Among the possible causes of idiopathic osteoporosis there is the oxidative stress, caused by the unbalancing between production of free radicals, molecules characterized by high reactivity due to one or more unpaired electrons in the external orbital, and antioxidant defenses in the biological systems, which are under a strict hormonal control[30].

Reactive oxygen species (ROS) are the most important and studied of the free radical species. Their *in vivo* production occurs especially during physiological oxidative processes of energetic substrates in the mitochondrial respiratory chain[31,32]. Other important kinds of free radicals are nitrogen reactive species[33].

In special conditions, such as obesity, the energetic demand is augmented and this increases the electronic flow in the respiratory chain producing ROS[34].

An over-production of free radicals is connected to several pathological conditions, such as cardiovascular and rheumatological diseases[31], and more in general ROS damage occurs in any inflamed tissues, causing cellular lysis and intracellular content release.

There are different defensive mechanisms against the production and the effects of free radicals[31]. They act both in the endoplasmic network (mitochondria, plasmatic membrane, peroxisomes and cytosol) and in the extracellular ambient.

The first mechanism is the avoiding of production or the quick inactivation of free radicals, thanks to the action of enzymes like catalase, peroxidase glutathion complex and superoxydedismutase (SOD), or of transition-metal binding proteins like transferrin, ferritin and ceruloplasmin.

The second mechanism uses intermediate radicals, called "scavengers", to stop the spread of the lipid peroxidation chain. The scavengers can be water-soluble, such as albumin, bilirubin, ascorbic acid, urates and thiols, or liposoluble, such as vitamin E and coenzyme Q_{10}, which is the only liposoluble antioxidant synthesized in living organisms. The mobility of these molecules, particularly the liposoluble ones and especially at membrane level, allows to intercept radicals and transform them into more stable molecules stopping radical chain.

The last mechanism consists in removing molecules damaged by oxidative attack, allowing the reconstitution of normal structures (e.g. specific phospholypases remove the peroxidized fatty acids, making possible the re-acylation of damaged molecule by an acyl-CoA and the respective enzyme)[31].

Some data suggest that OS can lead to structural and functional cartilage damages like cell death and matrix degradation, as in inflammatory diseases[35]. It is know that OS is present in ageing per se and ageing-related disorders[36]. OS has been considered the determinant of aging and lifespan[37,38].

Recent data in rodents indicate that ageing and the associated ROS increase are the main mechanism underlying osteoporosis both in males and females. Molecular mechanisms have been investigated, especially the FoxOs and β-cathenin. FoxOs, a subclass of a large family of transcription factors characterized by a DNA binding domain called Forkhead box, is an important resource against OS. They regulate the transcription of antioxidant enzymes such as Mn-superoxide dismutase (SOD) and catalase as well as genes involved in cell cycle, DNA repair and lifespan[39,40]. Moreover, ROS-activated FoxOs in early mesenchimal progenitors also divert β-cathenin away from signaling of Wnt glycoprotein signaling, causing decreased osteoblastogenesis. Lipid oxidation is another phenomenon which, through generation of ligands activating peroxisome proliferators-activated receptor γ and attenuating Wnt signaling, contributing to the decrease of bone formation[15].

Many studies are centered on the role of steroids, especially estrogens[41-47]. However, the two theories (based on OS, on one side, and the central role of estrogens, the so called "estrocentric" hypothesis), could be unified considering the ability of estrogens in modulating endogenous antioxidant systems[30].

Our study, perfomed in humans *in vivo*, shows that LAG values are reduced in osteoporotic patients, in comparison with age-matched subjects and with middle-aged osteoporotic subjects. This should be a condition of reduced antioxidant defence and therefore increased risk for oxidative damage. We excluded patients with secondary osteoporosis due to endocrine disturbances, in particular hypogonadism and growth hormone deficiency, which are known to be associated with OS[30,48].

We cannot explain why, on the contrary, in middle-aged patients, LAG showed a greater value, although non significant, in comparison with age-matched controls; we hypothesize that in a condition of increased OS, antioxidant system increase as a compensatory mechanism, as suggested in other clinical models; however, this mechanism could be defective in ageing patients.

Another question is whether OS is cause or consequence of osteoporosis. This is now considered as a systemic disease, but OS could cause an interference with Vitamin D metabolism and/or action. Further studies could clarify these mechanisms.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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Introduction: Transient Osteoporosis of Hip is a benign, acute onset, self-limiting disorder of unknown aetiology commonly seen in middle aged men and pregnant females. Case Report: We report a case of acute onset sharp pain in right hip in a physician without history of antecedent trauma. The diagnosis of Transient Osteoporosis of Right Hip was made based on clinical and MRI findings. The probable cause can be attributed to long standing working condition. There was complete resolution of symptoms by conservative treatment in the form of analgesics and non-weight bearing. Conclusion: High lev... Objective. To evaluate the results of surgical correction of idiopathic scoliosis in patients aged 18â€“50 years, in terms of different age groups. Material and Methods. A total of 393 patients (348 women, 45 men) with idiopathic scoliosis were operated on during 1996â€“2015. In all cases, posterior correction of the deformity was performed using segmental third-generation instrumentation. The primary curve and structural countercurve were included in the fusion area. Hook fixation was performed in 298 cases, and hybrid (hooks and pedicle screws) in 95. The period of postoperative follow-up was 4. Types of primary male osteoporosis include age-related osteoporosis and idiopathic male osteoporosis. Age-related osteoporosis in men, like in women, is more likely to occur as age increases, and is typically seen in males over the age of 70 years.Â In privately insured patients, the mean incremental direct cost per patient with nonvertebral fractures compared to control patients without fractures was US$6,888 (US$13,446 versus US$6,558; P<0.05), of which, 35% was composed of inpatient costs and 15% was composed of medication costs.62 In the same study, the cost to Medicare for nonvertebral fractures. Osteoporosis develops in older adults when the normal processes of bone formation and resorption become uncoupled or unbalanced, resulting in bone loss. Fractures are the result of decreased bone mass and strength, and, in the case of wrist and hip fractures, they usually involve a fall. Osteoporosis prevention and treatment programs should therefore focus on strategies that minimize bone resorption and maximize bone formation, as well as on strategies that reduce falls.Â Calcium and vitamin D are required for bone health at all ages. In order to maintain a positive calcium balance, the current recommendations for calcium intake for postmenopausal women and men aged 65 years and older is at least 1200 mg per day of elemental calcium.