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Endogenous Sex Steroid Levels in Women with Generalised Osteoarthritis

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Summary **Epidemiologic and clinical observations have suggested a relationship between generalised osteoarthritis (GOA) and hormonal and menopausal factors in women. We explored the hypothesis that postmenopausal women with GOA have altered sex hormone status compared with control women. We studied 112 women (mean age 64) with GOA. Controls were 151 women (mean age 54) from the general population without clinical evidence of hand or knee OA. All women were postmenopausal. Serum was assayed by RIA for testosterone, oestradiol, sex hormone binding globulin (SHBG), and dyhydroepiandrosterone sulphate (DHEAS). Because of the differences in mean ages, the results were compared according to equal age groups divided on the basis of tertiles. SHBG was lower in the GOA group, reaching significance in the middle group 53-61 years (58.0 vs 67.9nmol/l $p<0.05$). Testosterone was slightly higher in GOA women aged under 53. No consistent differences were seen in the older age group or for the other sex steroids. These preliminary data suggest that middle-aged women with GOA have lower circulating SHBG levels. This implies that higher circulating free oestrogens and androgens are present suggesting a role in the aetiopathogenesis of GOA.**

Key words: **Osteoarthritis, Hormones, Sex Hormone Binding Globulin.**

INTRODUCTION

Osteoarthritis (OA) is a group of clinically heterogeneous disorders unified by the pathological features of hyaline cartilage loss and subchondral bone reaction. OA in women more commonly affects multiple joints and is usually more severe than in men (1). The apparent prominence of women presenting with polyarticular symptoms in middle-age, has fuelled speculation that there may be some relationship between the onset of OA and the menopause. Kellgren and Moore described a form of "menopausal arthritis" in a group of women with Heberden's nodes characterised by a rapid onset of symptoms and multiple joint involvement (hands, spine and knees) and renamed the condition "primary generalized osteoarthritis" (2). A few small studies have examined urinary and serum sex-hormone levels in OA women with inconsistent results (3-5). This might in part be explained by the heterogenous groups of patients studied or the variable nature of oestrogen levels or oestrogen receptor sensitivity. Because of the possibility that hormonal changes may influence the disease, we studied the levels of several serum sex steroids in postmenopausal women with GOA.

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METHODS

Cases

Subjects consisted of 112 female outpatient attenders with a diagnosis of GOA, as defined by the presence of clinical and radiological evidence of OA in the hands and knees with clinical evidence of Heberden's nodes.

Controls

Controls were 151 women derived from the general population who had all been examined by a rheumatologist and did not demonstrate any overt clinical evidence of osteoarthritis. The majority of the women were attending a "well-woman" screening clinic. The data on the cases and controls had been collected independently as part of initially separate projects. However, all women had serum samples taken and stored under identical

conditions for simultaneous assay. All women reported were postmenopausal. This status was confirmed biochemically by levels of serum oestradiol and these levels were used as a definition of postmenopausal status in all women. Individuals who had taken any form of hormone replacement therapy were excluded.

Hormonal analysis

A serum sample was collected from the women for sex hormone estimation. Blood was collected between 9 am and 7 pm, the serum was immediately stored at -20 degrees before assay. Radioimmunoassay was used to measure levels of oestradiol, sex hormone binding globulin (SHBG), testosterone, and dehydroepiandrosterone sulphate (DHEAS) and has been previously described (6). The assay for testosterone involved solvent extraction (with diethyl ether) followed by radio-immunoassay using ^{125}I Iodine tracer. A rabbit antiserum was raised to a conjugate of testosterone-3-CMO-BSA. Separation of antibody-bound and free hybrid was achieved with dextran-coated charcoal suspension. SHBG was measured using a saturation analysis technique. The principal involved is that a fixed amount of tritiated dihydrotestosterone (DHT) and non-tritiated DHT is added to all samples, standards and quality controls. At equilibrium the bound DHT is precipitated with saturated ammonium sulphate. This method is outlined in more detail elsewhere (7). The within and between precision of the assays between 8 and 12%, in our laboratories. Women were included for further analysis if serum oestradiol levels were within the postmenopausal range (<100pmol/l).

Statistical analysis was made by comparing mean hormone levels of the two groups by Student t-test using logarithmic transformations when the distribution was non-normal. Adjustment for age differences was made by stratifying the two groups into three age-group tertiles on the basis of age distribution.

RESULTS

The mean ages of the GOA women were greater than the controls, namely 64.3 compared to 53.7 years. The minimum age was 41 and the maximum 81. The mean levels of the sex steroids are given in Table I. Overall levels of testosterone were significantly greater in the GOA group, and DHEAS and SHBG levels were lower, although the latter failed to reach significance. No difference was noted for serum oestradiol. Because of the differences in age between the groups, women were divided into 3 approximately equal age-group tertiles obtained from the age distribution of all women. The results are given in Table II and the mean levels for each sex steroid are given for each age group. Below the age of 53, testosterone levels were significantly higher, and SHBG slightly lower than in controls. For women aged 53-61 no differences were seen for testosterone, but SHBG levels were significantly lower 58.0 nmol/l versus 67.9 nmol/l. For the oldest age group, no consistent differences for any of the sex steroids was seen. To test whether using other arbitrary age-groupings might produce different results, the women were also stratified into three groups aged 40-49, 50-59, and 60+ rather than tertiles. The results were very similar with SHBG being significantly lower in the middle group aged 50-59 [44.9 (18.5) versus 67.1 (26.1) nmol/l, $p<0.01$].

Weight recordings were available on 152 individuals. Although as expected there was a weak overall inverse correlation between weight and SHBG levels ($r=0.23$, $p<0.05$), in the individuals studied there was no marked difference in weight (GOA means 66.4 kg, $n=35$ versus

Table I: *Sex steroids in women with generalised osteoarthritis and controls*

	GOA mean (SD)	Controls mean (SD)	p value
Number	112	151	
Age (yrs)	64.3 (7.6)	53.7 (6.0)	0.001
Testosterone (μg/ml)	2.2 (0.6)	1.9 (0.6)	0.001
SHBG (nmol/L)	61.0 (22.4)	65.5 (25.4)	0.1
Oestradiol (pmol/l)	27.3 (16.0)	25.2 (13.1)	0.28
DHEAS (μmol/l)	2.7 (1.5)	3.2 (2.2)	0.03

Table II: *Mean sex steroid levels by age group*

	GOA mean (SD)	Controls mean (SD)	p value
Age group 41-52 (n=77)	**(n=8)**	**(n=69)**	
Age (yrs)	48.2 (2.9)	49.3 (2.6)	0.31
Testosterone (pmol/L)	2.5 (0.5)	1.9 (0.6)	0.01*
SHBG (nmol/L)	58.0 (20.7)	68.0 (24.9)	0.28
DHEAS (μmol/L)	3.2 (1.0)	3.5 (2.4)	0.50
Oestradiol (pmol/L)	27.4 (15.1)	24.5 (11.2)	0.72
Age Group 53-61 (n=95)	**(n=28)**	**(n=67)**	
Age (yrs)	58.3 (3.0)	55.1 (2.6)	<0.05*
Testosterone (pmol/L)	1.9 (0.5)	1.8 (0.5)	0.60
SHBG (nmol/L)	58.0 (20.7)	67.9 (24.9)	<0.05*
DHEAS (μmol/L)	3.2 (1.8)	3.0 (1.9)	0.30
Oestradiol (pmol/L)	24.9 (13.7)	26.1 (15.0)	0.56
Age Group 62-81 (n=91)	**(n=76)**	**(n=15)**	
Age (yrs)	68.2 (5.1)	67.5 (4.9)	0.60
Testosterone (pmol/L)	2.3 (0.6)	2.3 (0.5)	0.74
SHBG (nmol/L)	65.2 (22.2)	62.9 (24.2)	0.34
DHEAS (μmol/L)	2.4 (1.3)	2.3 (1.4)	0.51
Oestradiol (pmol/L)	28.1 (17.0)	24.7 (13.2)	0.80

* Statistically significant

66.8 kg, n = 117 for controls). When divided into two weight categories based around the median, SHBG levels remained lower in the OA cases in both weight categories; 64.5nmol/l (n = 17) versus 69.9nmol/l (n = 57) for the lightest women, and 61.0 nmol/l (n = 21) versus 65.3 nmol/l (n = 74) for the heaviest women. Thus basic stratification by weight groups produced similar results to the crude overall comparison. Unfortunately weight recordings were scanty in the middle age-group, although the 5 cases available had a slightly higher mean weight compared to the 55 controls (69.4 kg versus 67.2kg).

DISCUSSION

The data suggest that abnormalities of the sex steroids exist in women with GOA aged 60 or under. The major differences when age is accounted for is in lower levels of SHBG and slightly higher testosterone levels in the youngest subjects. No obvious differences for any of the hormones were seen in those aged over 61. The actual differences in mean testosterone levels, although significant were relatively small compared to the differences in SHBG which is likely to be of greater physiologic importance.

Before considering possible implications of these findings, certain limitations of the data should be discussed. First by using prevalent cases, true cause and effect cannot be distinghuished, and changes due to lifestyle or treatment could have affected the results. Moreover, as the date of onset of OA cannot be accurately ascertained, disease duration and its effect on hormones could not be assessed. Age differences between the groups were apparent, and although stratification was used to adjust for this, the age strata were unbalanced in terms of numbers, because as might be expected there were more elderly GOA patients and fewer "normal" control subjects. As different methods of age-grouping produced consistent results, we believe the results are unlikely to be due to differences in age distribution. No hormonal differences were noted for the more elderly subjects, which might be expected if hormonal differences are more likely to influence the onset of the disease rather than the course later in life.

For some of the sex steroids, notably the oestrogens, obesity can be an important factor in regulation of endogenous serum levels. Unfortunately, weight recordings were not available on the majority of subjects. However for the 169 in whom data was available, no major differences in weight were evident which could have explained the differences in SHBG levels. Furthermore, when divided into two weight groups, those with GOA still had lower levels of SHBG and higher testosterone levels in both high and low weight groups. Although small weight differences between OA and controls could have occurred and would have been expected given the relationship between OA and obesity, the differences in hormonal status were unlikely to have been explained solely by differences in body weight.

In any study where a number of variables are compared there is a chance that a significant finding may arise by chance, especially within subgroups. However, the difference found between SHBG levels in the postmenopausal women was large and unlikely to have occurred by chance. In addition our "a priori" hypothesis was that endogenous hormone levels were higher in women with GOA, and therefore the statistical tests used were appropriate. The use of oestradiol levels to define menopausal status was necessary due to the lack of verifiable recalled information or gonadotrophin levels. This may have resulted in some misclassification in the youngest category, although both cases and controls were treated in the same way and this is unlikely to have distorted the results.

There is good epidemiologic and clinical evidence to link GOA with hormonal factors. In the population surveys of Lawrence, X-ray evidence of nodal GOA was three times higher in women in the age range 45-64 (1). Other hospital-based studies have shown a female to male ratio as high as 10:1, with a marked peak of age of onset at 50 years in women for nodal GOA (8). Joint symptoms are also one of the principal components of the climacteric (9), and may be associated with higher oestradiol levels (10). Other studies have found increased rates of hysterectomy in women developing OA suggesting hormonal differences (11). Obesity has been found to correlate in females with generalised and knee OA and also for a number of non-weight bearing sites (12). It appears to be related to excess body weight rather than muscle bulk (13). Obesity in women is a known cause of hyperoestrogenism via the peripheral formation of oestrogen from androstenedione in the fat tissues. After the menopause this route becomes the principal source of oestrogens. Thus, the association between OA and obesity also suggests a role of endocrine influences in the development of OA.

This hypothesis is supported by some animal models which show a worsening of lesions with oestrogens and improvement with tamoxifen (14). Oestrogen may act on subchondral bone and cartilage receptors via receptors and second messengers such as the regulatory polypeptides TGF-β or cartilage-inducing factor-A, interfering with osteoclast and osteoblast coupling and cartilage turnover (15).

A number of workers have studied urinary and serum sex-hormone levels in women with existing OA, however, the results have been inconsistent (3-5). The present study was much larger than any previous one, and looked at a more homogenous group of women. The finding of lower SHBG levels in GOA women under 61 years of age, has a number of interpretations. Abnormalities of SHBG have also been seen in diseases with a hormonal basis such as breast cancer or osteoporosis. First it implies that the levels of "free" circulating oestrogen and testosterone were higher in those women. This is because most of the oestradiol and testosterone carried in the circulation is bound either to SHBG or albumin, which act as carrier proteins and regulate the levels of active hormone available to the target tissues. Another possibility is that the difference in SHBG is acting as a marker for certain growth factors, notably Insulin-like growth factor I, and its binding proteins BP-1 and BP-2, as well as Insulin itself. These have been implicated in the control of bone growth, mediating the actions of growth hormone, as well as a number of other roles at different sites (16). Correlations between SHBG and levels of circulating BP-1 have also been reported (17). The lack of difference in oestradiol which is the major active form of the oestrogens, might be explained by the relatively low sensitivity of the assay (>20pmol/l) which makes a high proportion of postmenopausal samples undetectable. In addition, in this age group oestrone which is the main storage form of oestrogen was not measured. However, higher levels of testosterone imply that raised levels of the precursors of oestrone were present.

In summary, these findings suggest that postmenopausal women under 60 with GOA have higher levels of circulating free sex hormones, which may have an aetiological or pathogenetic role. These results need confirmation, ideally using greater numbers of women aged 45-60 where perhaps levels of other hormones as well as growth factors can be assessed.

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