Differences in androgens of HIV positive patients with and without Kaposi sarcoma

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Abstract

Aim—Since most forms of Kaposi sarcoma are much more common in men than in women, the aim of this study was to examine serum concentrations of sex steroids in HIV positive men with and without Kaposi sarcoma.

Methods—Blood samples from 34 HIV positive men without Kaposi sarcoma (KS−) and 28 with Kaposi sarcoma (KS+) and from 35 HIV negative men (controls) were analysed for adrenal and gonadal steroids. Further analysis was done in subgroups classified by CD4 lymphocyte counts.

Results—KS+ patients had significantly higher serum dehydroepiandrosterone (DHEA) and testosterone concentrations than the KS− patients, and their DHEA, DHEA sulphate, testosterone, and androstenedione values were higher than in the controls. The KS+ patients with more than 500 CD4 lymphocytes per mm³ had significantly higher serum DHEA, DHEA sulphate, and testosterone than the KS− patients with the same CD4 counts; those with 500–200 CD4 cells/mm³ had higher serum DHEA and testosterone than the equivalent KS− men; and those with <200 CD4 cells/mm³ had raised DHEA only compared with KS− men. Both KS+ and KS− men had higher serum progesterone and oestradiol than the controls. Glucocorticoids were not significantly altered.

Conclusions—The high androgen levels in KS+ patients, particularly in the early stages of the disease (>500 CD4 cells/mm³), may affect the immune system by inducing an abnormal cytokine profile, or by increasing T8 proliferation and activation, or both. This raises the question of the relationship between androgens and Kaposi sarcoma.

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Keywords: Kaposi sarcoma, HIV infection, androgens, DHEA, steroid hormones, immune system.

Most forms of Kaposi sarcoma are much more common in men than in women—the ratio is 10 to 15:1 for the classic and African forms.¹ The epidemic form of Kaposi sarcoma, which is associated with HIV infection, is also exceptionally common among male patients,^{1,2} and homosexual and bisexual men are 10 times more at risk.³ Certain factors favour the development of Kaposi lesions. Various cytokines,

particularly IL6, can act as autocrine/paracrine growth factor for AIDS associated Kaposi sarcoma.^{4,5} Glucocorticoids also favour the formation of Kaposi lesions.⁶ There have been reports⁷ of Kaposi sarcoma developing during the treatment of HIV patients with corticoids. The incidence of this condition began to increase with the use of immunosuppressive therapy for transplant recipients.⁸ Experimental studies also show that lesions resembling those of Kaposi sarcoma develop in male transgenic mice, but not in females.⁹

There is now general agreement that the steroid hormone profile of patients infected with HIV changes during the course of the viral infection.^{10–14} The serum concentrations of cortisol are normal or slightly high at all stages of HIV infection.^{11,13,14} By contrast, androgen levels appear to be closely correlated with the stage of HIV infection.¹⁴ Asymptomatic HIV positive men (stages II and III) in general have high serum concentrations of androgens (dehydroepiandrosterone (DHEA), androstenedione, testosterone), whereas those with AIDS have subnormal androgen concentrations. There is also evidence for a relationship between CD4 count and serum DHEA or DHEA sulphate values in HIV infected men at risk of progression to AIDS.¹⁵ These changes in hormone concentrations, particularly of the androgen profile, during the course of HIV infection might result in significant alterations in immune competence, especially cytokine profiles and T cell activity and function.^{16,17} Androgens, such as DHEA and DHEA sulphate, enhance in vivo the capacity of activated helper T cell to produce IL-2,¹⁸ even in the presence of glucocorticoids,¹⁸ which inhibit IL-2 production.¹⁸ The production of IL-2 is decreased in subjects with progression of HIV-1 infection.¹⁹ DHEA and DHEA sulphate supplements appear to correct the age associated dysregulated production of T cell lymphokines.²⁰ The differentiation of T lymphocytes into helper cells (T4) or suppressor (T8) depends on the concentrations of androgen.^{16,21} Testosterone stimulates the activity and function of T suppressor cells.²² Other data indicate that adult males who are very responsive to androgens produce fewer antibodies.²³

These epidemiological, clinical, and experimental data suggest that the steroid hormone environment and the immune status of the host may influence the risk of Kaposi sarcoma associated with HIV infection. We therefore studied the serum concentrations of corticoids, progestins, androgens, and oes-

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Table 1 Clinical information on HIV positive men classified according to the CD4 lymphocyte count

Group	CD4 range (count/mm ³)	Number of subjects	Age (years)	Time since diagnosis of HIV (months)	Previous infections	Previous treatment	CD4 count (per mm ³)	CD8 count (per mm ³)
KS-	>500	n=7	37 (28-50)	26 (15-32)	0	0	944 (781-1366)	1024 (557-1733)
KS+	>500	n=5	39 (26-62)	6 (0-23)	0	0	893 (563-1057)	772 (450-1246)
KS-	500-200	n=16	42 (29-63)	42 (0-78)	5	3	329 (209-444)	1206 (309-2174)
KS+	500-200	n=12	36 (23-45)	24 (0-43)	2	3	310 (211-422)	817 (251-2062)
KS-	<200	n=11	38 (30-44)	60 (4-102)	2	8	89 (3-169)	565 (211-879)
KS+	<200	n=11	40 (30-44)	48 (0-78)	2	8	107 (10-194)	966 (239-2208)

KS- = HIV positive men without Kaposi sarcoma; KS+ = HIV positive men with Kaposi sarcoma

trogens in male HIV positive patients with and without Kaposi sarcoma and in HIV negative men. We also determined the steroid hormone concentrations of HIV positive patients with and without Kaposi sarcoma classified according to their absolute CD4 lymphocyte count. These data were analysed to determine if there was any relationship between endogenous serum steroid hormone concentration and the presence of Kaposi sarcoma in a cohort of HIV positive men.

Methods

PATIENTS

Serum samples from Kaposi sarcoma negative and positive patients in each CD4 range were selected under double blind conditions, according to a single criterion—that the two groups of patients were in the same clinical condition at the time the samples were taken. We then examined the records of the patients for risk factors, age, previous infection, and treatment. All samples were taken from HIV positive patients at the Rothschild hospital (University Paris VI). Twenty eight were HIV positive men with Kaposi sarcoma (group KS+) and 34 were HIV positive men without Kaposi sarcoma (group KS-). HIV infection was confirmed by western blot. They were classified according to their CD4 lymphocyte counts. The patient data are shown in table 1. The KS+ group included five patients with >500 CD4 cells/mm³, 12 patients with 500-200 CD4 cells/mm³, and 11 patients with <200 CD4 cells/mm³. The KS- group included seven patients with >500 cells/mm³, 16 patients with 500-200 cells/mm³, and 11 patients with <200 cells/mm³. Two of the KS+ patients were examined twice (first in group 500-200, and then in the group <200 CD4), as they suffered from a recurrence of Kaposi sarcoma after four years of remission. The KS+ patient samples were taken within three months of Kaposi sarcoma being diagnosed. All except five were homosexual. In one KS+ patient and two KS- patients the risk factor was unknown, one KS- patient was a drug addict, and one KS- patient had been transfused. The two groups were of similar age, and had similar previous opportunistic infections and therapy. Only the mean duration of known HIV infection differed; this was because the KS+ patients were seen much earlier than the KS- patients.

The CD4 cell count indicated that patients with >500 CD4 lymphocytes had had no opportunistic infections or treatment; in the 500-200 CD4 range, KS+ patients had no opportunistic infections but the KS- patients included one who had had tuberculosis, one with ocular toxoplasmosis, and one with gastric lymphoma. KS+ patients with <200 CD4 lymphocytes included one with toxoplasmosis, one with cytomegalovirus (CMV) retinitis, and two with previous Kaposi sarcomas; KS- patients with <200 CD4 lymphocytes included one with toxoplasmosis, one with multifocal leucoencephalitis, and one with a lymphoma. Antiviral therapy was given as follows: in the 500-200 CD4 cell group, one KS+ patient had taken zidovudine, and two had taken imuthiol; two KS- patients had taken zidovudine and one had taken imuthiol; in the <200 CD4 cell group, six KS+ patients had been given antiviral treatment (four zidovudine, two zidovudine followed by DDI), two had taken interferon α 2b four years earlier; seven KS- patients had had zidovudine and two had had imuthiol. Five KS+ and six KS- patients had primary prophylactic pneumocystosis. Patients were not treated with ketoconazol, glucocorticoids, or interferon at the time the western blots were taken.

The control group consisted of 35 HIV negative male blood donors aged 18-50 years.

BLOOD SAMPLES

Blood samples were collected between 0800 and 1000 and allowed to coagulate before separation of serum by centrifugation (3000 rpm/10 minutes at 4°C). Serum samples were stored at -20°C until assayed.

STEROID EXTRACTION AND CHROMATOGRAPHIC FRACTIONATION

(1) Serum samples (1 ml) were extracted for 30 min with 5 ml of organic solvent (ethyl acetate/cyclohexane, 1/1) and the aqueous phase was removed by freezing (-20°C). The organic phase was evaporated to dryness, taken up in 1 ml of solvent system I (benzene/ethanol, 95/5) and placed on a Sephadex LH20 microcolumn (0.5 × 6 cm). Progesterone and androstenedione were first eluted with 2.6 ml of solvent I. Oestrone was then eluted with 3.5 ml

of solvent I followed by 1.0 ml of solvent II (benzene/ethanol, 90/10). Finally, oestradiol and cortisol were eluted with 6 ml of solvent II.

(2) Serum samples (0.5 ml) were extracted as above and placed on Sephadex LH20 micro-columns: testosterone and 17α hydroxyprogesterone (17α OH-progesterone) were eluted with 5.5 ml of solvent I.

(3) A third series of serum samples (0.5 ml) was extracted with organic solvent (ethyl acetate/cyclohexane, 1/1) for direct radioimmunoassay (RIA) of dehydroepiandrosterone (DHEA).

(4) DHEA sulphate concentrations were determined directly without extraction. Samples were diluted with radioimmunoassay buffer for RIA.

The yields from these extraction and purification steps were between 70% and 95%.

RADIOIMMUNOASSAY OF STEROIDS

Oestrone, oestradiol, progesterone, 17α OH-progesterone, cortisol, testosterone, androstenedione, DHEA, and DHEA sulphate were assayed using rabbit antisera from Miles, Yeda, Israel (anti-E1 6-thyroglobulin serum, anti- 17β -oestradiol-6-BSA serum, anti- 17α OH-progesterone-7-BSA serum, anti-F-21 thyroglobulin serum, anti-testosterone-7- α -BSA serum, and anti-androstenedione 7- α -BSA serum) and rabbit antisera from Biosys, France (anti-progesterone-11HS-BSA, anti-DHEA 15-CH₂-CO-BSA, anti-DHEA sulphate 7- β -CM-BSA). The detection limit was 18 pmol/l in all cases. All hormones were assayed as described in ¹⁴. DHEA and DHEA sulphate were determined by RIA (Biomérieux kits, Chamberlins-les-Bains, France).

The tritiated steroids $2,4,6,7^3\text{H}$ oestrone (107 Ci/mmol), $2,4,6,7^3\text{H}$ oestradiol (99 Ci/mmol), $1,2,6,7^3\text{H}$ progesterone (82 Ci/mmol), $1,2,6,7^3\text{H}$ 17α OH progesterone (89 Ci/mmol), $1,2,6,7^3\text{H}$ cortisol (85 Ci/mmol), $1,2,6,7^3\text{H}$ testosterone (94.1 Ci/mmol), $1,2,6,7^3\text{H}$ androstenedione (96 Ci/mmol), $1,2,6,7^3\text{H}$ DHEA (86.6 Ci/mmol), and $1,2,6,7^3\text{H}$ DHEA sulphate (76.8 Ci/mmol) were purchased from the Radiochemical Centre, Amersham. All were 99% pure; purity was checked by thin layer chromatography. Radioactivity was determined on samples dissolved in 4 ml Opti-Fluor (Packard) by counting in a Packard 1500 liquid scintillation analyser using the internal standard for quench correction.

STATISTICAL ANALYSIS

The data were analysed by the Wilcoxon non-parametric test and the Student-Newman-Keuls multiple range test. The differences were considered significant at probability (p) values of <0.05 .

Results

STEROID HORMONE CONCENTRATIONS OF HIV POSITIVE (WITH AND WITHOUT KAPOSI SARCOMA) AND HIV NEGATIVE MEN

The hormone concentrations of HIV negative men (control) and all the members of the two

groups of HIV positive men, with and without Kaposi sarcoma, were compared.

Progesterins and cortisol—Serum progesterone (fig 1) in the KS+ and the KS- groups was higher (125%, $p<0.01$, and 105%, $p<0.01$, respectively) than in the controls. There was no difference between KS+ and KS- patients.

Figure 1 Serum progesterone concentrations in HIV positive men with (KS+) or without (KS-) Kaposi sarcoma and in HIV negative men (controls). Concentrations were determined by radioimmunoassay. Control, $n=35$ subjects; KS-, $n=34$ subjects; KS+, $n=28$ subjects. Error bars = SEM. KS- v control $p<0.01$; KS+ v control $p<0.01$; KS+ v KS-: NS.

Figure 2 Serum androgens [dehydroepiandrosterone (DHEA), DHEA sulphate, androstenedione ($\Delta 4$), and testosterone (T)] in HIV positive men with (KS+) or without (KS-) Kaposi sarcoma and in HIV negative men (controls). Concentrations were determined by radioimmunoassay. Control, $n=35$ subjects; KS-, $n=34$ subjects; KS+, $n=28$ subjects. Error bars = SEM. KS- v control: DHEA sulphate $p<0.05$; KS+ v control: DHEA, DHEA sulphate, T $p<0.01$, $\Delta 4$ $p<0.05$; KS+ v KS-: DHEA and T $p<0.01$.

Figure 3 Serum oestrone and oestradiol concentrations in HIV positive men with (KS+) or without (KS-) Kaposi sarcoma and in HIV negative men (controls). Concentrations were determined by radioimmunoassay. Control, $n = 35$ subjects; KS-, $n = 34$ subjects; KS+, $n = 28$ subjects. Error bars = SEM. KS- v control: E1 NS, E2 $p < 0.05$; KS+ v control: E1 NS, E2 $p < 0.05$; KS+ v KS-: E1 NS, E2 NS.

Serum cortisol and $17\alpha\text{OH}$ -progesterone concentrations in KS+ and KS- men were not significantly different from control. There was no difference between the KS+ group and the KS- group. The serum cortisol concentrations were: KS-, 380 (SEM 18.8) nmol/l; KS+, 360 (19.6) nmol/l; control, 337.6 (19.3) nmol/l. The serum $17\alpha\text{OH}$ -progesterone concentrations were: KS-, 3.5 (0.3) nmol/l; KS+, 4.24 (0.3) nmol/l; control, 3.64 (0.2) nmol/l.

Androgens—The serum concentrations of the androgens DHEA, DHEA sulphate, androstenedione, and testosterone in KS+, KS-, and controls are shown in fig 2.

The androgen concentrations were much higher in KS+ men than in controls: DHEA, DHEA sulphate, and testosterone were 60% above control values ($p < 0.01$) and androstenedione was 25% increased ($p < 0.05$). The only androgen that was significantly raised in KS- patients was DHEA sulphate (35%, $p < 0.05$). The DHEA and testosterone concentrations of KS+ patients were higher (85% and 40% respectively, $p < 0.01$) than those of KS- patients.

Oestrogens (fig 3)—The serum oestradiol concentrations in all groups of HIV positive patients were significantly higher (50%, $p < 0.05$) than in controls, but there was no difference between the KS+ and KS- subjects. The serum oestrone concentrations in HIV positive men were not significantly different from control. There was no difference between the KS+ and KS- men.

STEROID HORMONE CONCENTRATIONS OF HIV POSITIVE MEN ACCORDING TO THE CD4 LYMPHOCYTE COUNT

The hormone concentration of HIV positive patients with and without Kaposi sarcoma, classified according to their absolute CD4 lymphocyte counts, were compared.

Progestins and cortisol (table 2)—The progesterone concentrations were significantly low (30%, $p < 0.05$) only in KS+ patients in the 500–200 CD4 cells/mm³ stratum. The $17\alpha\text{OH}$ -progesterone concentrations were high (65%, $p < 0.05$) only in KS+ patients with > 500 CD4 lymphocytes/mm³. There was no difference in the cortisol concentrations of the two groups according to the CD4 lymphocyte count.

Androgens (fig 4)—Androgens were significantly higher in the KS+ men than in the corresponding KS- men in each of the CD4 lymphocyte count categories. These increases were as follows:

- (1) In the > 500 CD4 cell stratum, DHEA sulphate increased by 50% ($p < 0.02$), DHEA increased by 125% ($p < 0.02$), testosterone increased by 85% ($p < 0.02$), and androstenedione increased by 20% (NS).
- (2) In the 500–200 CD4 cell stratum, the DHEA and testosterone concentrations were 65% ($p < 0.01$) and 35% ($p < 0.05$) higher respectively than in the KS- patients.
- (3) In the < 200 CD4 stratum, only the DHEA concentrations were increased in the KS+ men (80%, $p < 0.05$).

Oestrogens (table 3)—The serum oestrone and oestradiol concentrations in the KS+ and KS- patients were not significantly different, regardless of the CD4 cell count.

Table 2 Serum progestin and cortisol levels in HIV positive men. Values are means (SEM)

Group	CD4 range (count/mm ³)	Number of subjects	Progesterone (nmol/l)	$17\alpha\text{OH}$ -progesterone (nmol/l)	Cortisol (nmol/l)
KS-	> 500	$n = 7$	1.02 (0.12)	2.42 (0.33)	392.0 (38.6)
KS+	> 500	$n = 5$	1.00 (0.2)	4.10 (0.60)*	401.6 (61)
KS-	500–200	$n = 16$	1.05 (0.11)	3.94 (0.45)	361.6 (22)
KS+	500–200	$n = 12$	0.70 (0.10)*	4.70 (0.60)	386.7 (32.6)
KS-	< 200	$n = 11$	0.94 (0.13)	3.64 (0.70)	382.5 (44.2)
KS+	< 200	$n = 11$	1.10 (0.15)	3.80 (0.50)	312.0 (28.07)

Serum progesterone, $17\alpha\text{OH}$ progesterone, and cortisol concentrations in HIV positive men with Kaposi sarcoma (KS+) or without Kaposi sarcoma (KS-), grouped according to their CD4 lymphocyte count, were determined by radioimmunoassay.

* $p < 0.05$ v KS-

Figure 4 Serum androgens [(A) dehydroepiandrosterone (DHEA) sulphate; (B) androstenedione ($\Delta 4$); (C) DHEA; (D) testosterone] in HIV positive men with (KS+) and without (KS-) Kaposi sarcoma. Concentrations were determined by radioimmunoassay. Error bars = SEM. The distribution of patients according to CD4 count is shown in table 1. >500 CD4, KS+ v KS-: DHEA sulphate, DHEA, testosterone $p < 0.01$, $\Delta 4$ NS; 500-200 CD4, KS+ v KS-: DHEA $p < 0.01$, testosterone $p < 0.05$; <200 CD4, KS+ v KS-: DHEA $p < 0.05$.

Table 3 Serum oestrogen concentrations in HIV positive men. Values are means (SEM)

Group	CD4 range (count/mm ³)	Number of subjects	Oestrone (pmol/l)	Oestradiol (pmol/l)
KS-	>500	n=7	111 (22)	205 (77)
KS+	>500	n=5	222 (78)	143 (29)
KS-	500-200	n=16	107 (18)	147 (18)
KS+	500-200	n=12	126 (33)	165 (15)
KS-	<200	n=11	152 (41)	187 (40)
KS+	<200	n=11	237 (55)	195 (22)

Serum oestrone and oestradiol concentrations in HIV positive men with Kaposi sarcoma (KS+) or without Kaposi sarcoma (KS-), grouped according to their CD4 lymphocyte count, were determined by radioimmunoassay.

Discussion

In this retrospective study we examined the serum concentrations of adrenal and gonadal steroids in HIV positive men with and without Kaposi sarcoma. All hormone assays and analyses were performed under double blind conditions, with the operator unaware of either the Kaposi status or the CD4 count. There was no difference between the KS+ and KS- patients in terms of age, mean CD4 count, opportunistic infections, or treatment. The results obtained suggest that only the serum concentrations of androgens in the two groups differed markedly. The differences in the other serum steroid hormone concentrations were either non-significant or inconsistent.

HIV positive patients with Kaposi sarcoma had significantly higher serum adrenal androgen (DHEA and DHEA sulphate) and gonadal androgen (testosterone) concentrations than the HIV positive men without Kaposi sarcoma, or the HIV negative men. This difference was still evident when the patients were classified according to their CD4 lymphocyte count. The serum DHEA and testosterone concentrations of the KS+ patients were higher than those of KS- patients, whatever the CD4 lymphocyte count. The increase in androgen levels was much greater in those KS+ subjects with more than 500 CD4 cells per mm³. In these patients with Kaposi sarcoma but without other clinical problems, all the androgen concentrations were higher than in HIV positive patients without Kaposi sarcoma. This difference was very significant for DHEA, DHEA sulphate and testosterone, but not significant for androstenedione, perhaps because of the small number of patients studied. High androgen levels have been reported in male subjects in the early stages of HIV infection (stages II and III according to CDC Atlanta criteria).¹⁴ The present study shows that the decrease in the absolute CD4 cell count and progression to AIDS is accompanied by a fall in the serum androgen concentrations. There is also a significant linear correlation between the CD4

cell count and the serum concentrations of DHEA sulphate only in the KS+ patients ($r = 0.4155$, $p < 0.01$). Both the DHEA and testosterone levels of KS+ subjects with 500-200 CD4 cells per mm^3 were higher than those of the KS- patients. But in the KS+ subjects with <200 CD4 cells/ mm^3 , only the DHEA concentration was higher than in KS- patients. A decrease in DHEA has been associated with increased risk of progression to AIDS in men with 200-499 CD4 cells/ mm^3 .¹⁵ A longitudinal study²⁴ indicates that there is a correlation between the decrease in serum DHEA in HIV-1 infected men and progression to AIDS. The association of other infections with Kaposi sarcoma could induce the fall in androgens, as seen in human septic and experimental endotoxin shock.^{25,26}

Studies on the relationship between the immune response and steroid hormones suggest that androgens regulate the immune system, influencing lymphocyte proliferation and function,^{17,21} and controlling the production of lymphokines.^{16,18} An excess of androgens should stimulate suppressor T cell function at the expense of helper T cell function.^{16,21} The adrenal androgens, DHEA and DHEA sulphate, enhance the capacity of activated T cells to produce interleukin-2 and γ -interferon, and reverse glucocorticoid induced inhibition of these important lymphokines. The high androgen levels observed in KS+ patients, particularly in early stages of the disease (with >500 CD4 cells/ mm^3), may affect the immune system by inducing an abnormal cytokine profile and increased CD8 proliferation.

Various clinical and epidemiological data have been associated with Kaposi sarcoma, for example, gender,^{1,2} age,² homosexuality or bisexuality,³ and immunosuppressive therapy.⁸ Whatever the exact mechanism by which Kaposi sarcoma develops, this report suggests that the clinical progression of Kaposi lesions is closely associated with the hormonal status, notably the androgen levels, of the patients. Further studies on the relationship between androgen concentrations, the cytokine profile, and T cell proliferation, activity, and function in HIV associated Kaposi sarcoma should indicate the exact position of androgens in the cause and effect interactions leading to this disease. Hence a clear understanding of the hormonal environment may provide a sound basis for the development of new therapeutic strategies.

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