

The Effects of Changes in Testosterone Level on the Development of Cytokines among Patients with Prostate Cancer

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Abstract

Objective: To study the mechanism of development of low-differentiated androgen-resistant prostate cancer

Patients and Methods: Fourteen patients with stage 3-4 cancer of the prostate were held under examination. Inspection was carried out before and 1-month after orchiectomy.

Results: One month after orchiectomy all patients showed a significant decrease in levels of testosterone ($p < 0.005$), 5α -dihydrotestosterone, 17β -estradiol ($p < 0.05$) in comparison to initial indicators, while levels of LH and FSH had increased ($p < 0.001$). The reduction of testosterone was accompanied by the increase of prolactin, STH, and estrone ($p < 0.05$). The decrease in the level of testosterone was accompanied by the increase in the levels of insulin, IGF-I ($p < 0.05$), bFGF ($p < 0.01$), 25-OHVitD_3 , and Ca^{++} ($p < 0.05$) as well as by a reduction in the levels of βTGF , IL- β , TNF α ($p < 0.05$), acid phosphatase ($p < 0.01$), alkaline phosphatase ($p < 0.05$) and PSA ($p < 0.005$).

Conclusion: After orchiectomy an increase in the level of factors that raised kariomitotic activity was observed, while a decrease in the level of factors regulating the cell cycle and beginning of apoptosis was observed.

Keywords: Prostate cancer, testosterone, main fibroblast growth factor, insulin-similar growth factor-1, insulin, transforming factor of growth- β

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INTRODUCTION

The androgen blockade method has been widely used during treatment of prostate cancer for more than 60 years.¹ Results of the androgen blockade and tumor regression are observed in 70-80% of patients.² However, a few years after the start of hormonal therapy the majority of patients shows the development of low-differentiated androgen-resistant prostate cancer.³

The probability of the development of prostate cancer significantly increases after forty years of age,⁴ from this period a decrease in circulating testosterone in the blood is observed in men. This decrease was named "partial androgen deficiency of aging men" (PADAM).⁵ PADAM provokes a breach of the mechanisms of regulation in the system of the gonadotropin-pituitary-hypothalamus, including an increase in the activity of the hypophysis,⁶ as well as an increase of 5 α -dihydrotestosterone and 17 β -estradiol.⁷ The given factors exert a significant influence on the development of prostate cancer.⁸

The goal of the work was to study the influence of the changes in the testosterone level on the levels of hormones and on cell growth factors in men. The study was conducted to prove the hypothesis that a significant reduction in the level of testosterone has a strong influence on the development of cancer of the prostate, including its low-differentiated hormonal-resistant form.

PATIENTS AND METHODS

Fourteen patients, age range 60-79 years, with stage 3-4 cancer of the prostate were studied. The presence of prostate cancer, the necessity of orchiectomy and a satisfactory mental and physical state served as criteria for inclusion into this study.

Patients were excluded from the study if they had any of the following: varicocele, changes in the function of the liver (transaminase and/or bilirubin higher than normal), or serum creatine higher than 0.11 mmol/l. Furthermore, patients who received medical treatment during the last three months with diuretics, antiandrogens, finasterid or permixon were also excluded.

All patients made up one group within which results of the tests were compared before and one month after orchiectomy.

Hormonal research and determination of the level of PSA

To determine the levels of LH, FSH, prolactin, and testosterone, kits made by "Alkor Bio" (Russia) were used. Levels of STH and IGF-1 were determined using kits made by BCM Diagnostic (USA). E2, PSA and insulin were determined using kits made by DPC (USA), 5 α -dihydrotestosterone with a kit by Alpha Diagnostic International (USA), estrone with a kit by DSLabs (USA), 25-OH VitD₃ by Biomedica, Be Gesellschaft mbh (Austria). bFGF, EGF, TGF- β were determined with a kit made by Cytimmune Sciences Inc. (USA), and IL-1 β , TNF α with kits made by "Scientific Research Institute OChB "Proteinovii Contour" (Russia). The minimal concentration of E2 which permitted a display of the method was 73.4 pmol/l.

Normal levels of LH range from 2.2-9.8 IU/l,⁹ FSH 1.0-11.8 IU/l, testosterone 13-33 nmol/l, prolactin 105-540 mIU/l, STH 0-264 pmol/l, normal levels of 5 α -dihydrotestosterone range from 0.86-3.4 nmol/l (according to the figures of the makers of the test-system), 17 β -estradiol in the blood plasma of men from 22-161 pmol/l,¹⁰ normal levels of PSA 0-4.0 ng/ml, insulin 43.0-193.7 pmol/l, IGF-1 40-280 mkg/l, TGF- β 19-71 ng/ml, IL-1 β 0-50 pg/ml, TNF α 0-50 pg/ml (according to the figures of the test-system makers).

Sensitivity and coefficients of the variations are as follows: for LH 0.5 IU/l and 8%; for FSH 0.5 IU/l and 8%; for prolactin 10 mIU/l and 8%; for STH 1.3 pmol/l and 8%; for testosterone 0.7 nmol/l and 8%; for 5 α -dihydrotestosterone 0.24 nmol/l and 11.7%; for 17 β -estradiol 0.1 pmol/l and 8%; for estrone 3.7 pmol/l and 7%; for PSA 0.01 ng/ml and 8%; for insulin 7.2 pmol/l and 10%; for 25-OH VitD₃ 1.5 nmol/l and 10%; for bFGF 0.49 ng/ml and 8%; for IGF-1 0.01 mkg/l and 7%; for EGF 0.1 pg/ml and 7%; for TGF- β 0.2 pg/ml and 7.9%; for IL-1 β 1 pg/ml and 5%, and for TNF α 1 pg/ml and 5%.

A step in this method was to draw blood in the morning, on an empty stomach, at a fixed time (08.00-10.00).^{11,12}

Determination of the activity of acid phosphatase, alkaline phosphatase and the level of Ca⁺⁺

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Randox (Great Britain). Normal indicators of acid phosphatase range from 0-5.7 E/l and for alkaline phosphatase 60-270 U/l. The normal level of Ca^{++} is 2.10-2.70 mmol/l. Sensitivity and coefficients of the variations constituted 0.33 E/l and 7.3% for acid phosphatase; 8.3 U/l and 5.9% for alkaline phosphatase. Sensitivity and coefficients of variations for Ca^{++} constituted 0.25 mmol/l and 1.8%.

Statistical analysis

The evaluation of results, before orchiectomy and one month after the operation, was done using the method of dispersion analysis of repeated measurements. All figures, in the text and in the tables, are represented in the form of average values and their standard deviations ($M \pm \delta$). Also shown are the average values of the parameters ($\bar{d}$), their standard errors (s_d) and the values of the Student criteria (t).¹³

RESULTS

In the initial test the average level of LH and FSH did not exceed normal values. The level of prolactin of all patients was within normal limit. The average

indicators of IL-1 β , acid phosphatase, alkaline phosphatase, TNF α , and PSA were increased, but testosterone and β TGF were decreased.

One month after the orchiectomy in comparison with initial data, a significant reduction of the levels of testosterone, 5 α -dihydrotestosterone and 17 β -estradiol was observed in all patients (on average 90.4%; 36.9% and 23.7% respectively), while an increase of LH and FSH was observed (on average 229% and 359% respectively). The reduction of testosterone was accompanied by a statistically significant increase of prolactin (on average 50.7%), STH (on average 46.6%) and of estrone (on average 69.9%). The decrease in 5 α -dihydrotestosterone determined the reduction of EGF (on average 68.3%).

The decrease in the level of testosterone was accompanied by a statistically significant increase of insulin (on average 53.7%), IGF-1 (on average 17.3%), bFGF (on average 43.9%), 25-OH Vit D₃ (on average 58.9%), Ca^{++} (on average 6.5%), and decrease of β TGF (on average 47.6%), IL-1 β (on average 58.7%), TNF α (on average 69.3%), acid phosphatase (on average 31.9%), alkaline phosphatase (on average 30.5%) and PSA (on average 81.1%) (Tables 1, 2, 3, 4).

Table 1 Levels of LH, FSH, prolactin, and STH before and one month after orchiectomy

	LH	FSH	Prolactin	STH
$\mu \pm \sigma$ before	7.2 $\pm$ 8.8	8.3 $\pm$ 11.8	219.7 $\pm$ 100.1	132.0 $\pm$ 189.2
$\mu \pm \sigma$ after	23.7 $\pm$ 14.1	38.1 $\pm$ 17.8	331.3 $\pm$ 220.4	193.6 $\pm$ 268.4
$\bar{d}$	16.5	29.8	111.6	61.6
s_d	3.4	3.9	49.5	26.4
t	4.907	7.678	2.253	2.450
	$p < 0.001$	$p < 0.001$	$p < 0.05$	$p < 0.05$

$N = 14$

Table 2 The levels of testosterone, DHT, E₂, estrone and PSA before and one month after orchiectomy

	Testosterone	DHT	E ₂	Estrone	PSA
$\mu \pm \sigma$ before	11.4 $\pm$ 8.9	2.333 $\pm$ 1.707	99.6 $\pm$ 36.8	2921.0 $\pm$ 3505.7	69.3 $\pm$ 59.3
$\mu \pm \sigma$ after	1.1 $\pm$ 0.7	1.472 $\pm$ 0.918	76.0 $\pm$ 8.0	4966.0 $\pm$ 6249.2	13.1 $\pm$ 22.5
$\bar{d}$	-10.3	-0.861	-23.6	2044.6	-56.2
s_d	2.5	0.364	10.3	887.9	15.3
t	4.150	2.367	2.287	2.303	3.671
	$p < 0.005$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.005$

$N = 14$

Table 3 The levels of insulin, bFGF, EGF, IGF-1 and β TGF before and one month after orchiectomy

	Insulin	bFGF	EGF	IGF-1	β TGF
$\mu \pm \sigma$ before	58.8 $\pm$ 22.2	26.9 $\pm$ 10.0	339.2 $\pm$ 199.9	184.0 $\pm$ 76.9	1748.5 $\pm$ 1271.4
$\mu \pm \sigma$ after	90.4 $\pm$ 53.8	38.7 $\pm$ 14.4	107.6 $\pm$ 154.2	215.9 $\pm$ 66.3	916.9 $\pm$ 664.3
$\bar{d}$	31.6	11.8	-231.6	31.9	-831.6
$s_{\bar{d}}$	12.2	3.5	104.7	12.8	366.8
t	2.531	3.356	2.213	2.489	2.267
	$p < 0.05$	$p < 0.01$	$p < 0.05$	$p < 0.05$	$p < 0.05$

N = 14

Table 4 Levels of 25OHD3, IL-1 β , TNF α , Acid phosphatase, Alkaline Phosphatase and Ca⁺⁺ before and one month after orchiectomy

	25-OHvitD	IL-1 β	FNO- α	Acid Phosphate	Alkaline Phosphate	Ca ⁺⁺
$\mu \pm \sigma$ before	43.2 $\pm$ 34.7	227.9 $\pm$ 216.3	75.5 $\pm$ 81.3	7.2 $\pm$ 3.7	548.8 $\pm$ 592.9	2.3 $\pm$ 0.1
$\mu \pm \sigma$ after	68.7 $\pm$ 95.5	94.1 $\pm$ 134.4	23.2 $\pm$ 12.0	4.9 $\pm$ 2.2	381.3 $\pm$ 439.2	2.5 $\pm$ 0.3
$\bar{d}$	25.5	-133.8	-52.3	-2.3	-167.5	0.1
$s_{\bar{d}}$	11.7	57.2	20.6	0.7	57.0	0.07
t	2.168	2.340	2.537	3.183	2.939	2.273
	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.01$	$p < 0.05$	$p < 0.05$

N = 14

DISCUSSION

The age-related reduction of testosterone, found in patients before orchiectomy, testifies to the fact that "partial androgen deficiency of aging men" (PADAM) accompanies the development of prostate cancer. A significant part of tissues have testosterone receptors. Testosterone takes part in the processes of growth and differentiation of cells.^{8,14} PADAM disrupts the natural cycle of development of cells containing androgen receptors. The given changes manifest themselves by a reduction in the intensity of reproduction of cells in an entire series of tissues as a proportion of aging.¹⁵⁻¹⁷ The transition of androgen-independent transitory-proliferation cells into the androgen-dependent pool of transforming cells,⁸ which requires, for further development, the presence of a physiologically necessary level of testosterone, is accompanied by a breach of the process of differentiation. The risk of their neoplastic transformation rises.^{17,18} An infringement of the development of the cells on the testosterone-dependent stage regularly hinders the development of the final stage of the cell cycle-apoptosis.

In order to make up for the inadequacy of the mytogenic action of testosterone, a whole set of compensatory-adaptation reactions is formed. These reactions touch on endocrinal, paracrine, and autocrine levels.¹⁷

In view of the interdependence of the neurohumoral regulatory processes,¹⁹ a reduction of the testosterone production is reflected on endocrinal regulation as a whole.

The reduced level of testosterone stimulates not only the secretion of LH, but also of gonadoliberein¹⁹ and FSH.

Upon the first test, the level of LH and FSH in most patients, despite the low level of testosterone, did not exceed the normal values. A month after orchiectomy, besides a reduction in the level of testosterone, 5 α -dihydrotestosterone, and 17 β -estradiol, all patients showed a significant increase of LH and FSH ($p < 0.001$).

The increased levels of testosterone and 17 β -estradiol repress the secretion of LH and FSH, as well as the secretion of gonadoliberein.¹⁹ Therefore, when the level of testosterone goes down there is a corres-

ponding response in the increase of the level of 5 α -dihydrotestosterone and 17 β -estradiol in men with PADAM.⁷ In some men there is no increase in the level of LH and FSH.

Thus it is essential to consider the increase of the levels of 5 α -dihydrotestosterone and 17 β -estradiol, equally to the increase in the level of LH, FSH and the reduction of the level of testosterone when diagnosing PADAM.

After orchiectomy an increase in the level of STH ($p < 0.05$) was observed depending on the stimulation of somatoliberin.¹⁹ An increase in the secretion of somatoliberin and repression of the secretion of somatostatin is observed during reduction of the sensitivity of the hypothalamic centers to inhibition with glucose. Insulin resistance promotes an increase of STH. After orchiectomy the level of insulin regularly increased ($p < 0.05$). Formation of STH is additionally stimulated by Vitamin D,^{14,17,19} the level of which increases after orchiectomy ($p < 0.05$).

After orchiectomy the level of prolactin continually increased ($p < 0.05$).

The fall in testosterone levels was accompanied by an increase in the activity of the aromatase. This confirms the increase of the estrone level ($p < 0.05$). Just like 17 β -estradiol, estrone is formed under the influence of aromatase.¹⁹ The formation of estrone from adrenal androgen-androstendion allows one to evaluate the activity of aromatase even when the level of testosterone is significantly reduced after orchiectomy.

Seeing that when the level of testosterone is decreased the activity of aromatase as well as of 5 α -reductase increases,⁷ the increase in activity of aromatase after orchiectomy points to the growth of 5 α -reductase activity.

Thus the reason for the decrease in the production of 5 α -dihydrotestosterone and 17 β -estradiol in patients following orchiectomy is that the level of testosterone decreases significantly (from which 5 α -dihydrotestosterone and 17 β -estradiol form). Despite this, the enzymatic activity, contrarily, increases.

An increase in aromatase activity may be promoted by prolactin,²⁰ IGF-1²¹⁻²³ insulin²⁴ and vitamin D,^{23,25} the levels of which regularly rose after orchiectomy ($p < 0.05$ for all three indicators).

The increase in the activity of aromatase and 5 α -reductase is determined by the physiological role of

estrogens and 5 α -dihydrotestosterone.

Estrogens induce an intensive metogenesis in the tissues containing specific receptors.²⁶ The estrogen receptors are located both in the cells of the stroma and in the epithelia of the prostate gland, with a primary localization in the stroma. The stimulation of growth of stromal cells induces a proliferation of the epithelia.⁸

5 α -dihydrotestosterone and testosterone, connecting with one and the same inner-cell receptor¹⁹, stimulate the proliferation activity of the cells.²⁷ The reduction of the levels and, accordingly, of the kariokynetic activity of testosterone ($p < 0.005$), 5 α -dihydrotestosterone ($p < 0.05$) (and, accordingly, of EFR ($p < 0.05$),²⁷ and 17 β -estradiol ($p < 0.05$) after orchiectomy is compensated not only by an increase in aromatase and 5 α -reductase activity, but also by an additional exertion of production by cells of peptidial growth factors. The given changes are conditional upon the endocrinal activators of reproduction.^{17,28}

In all patients after orchiectomy there ensued a reliable increase of the level of bFGF ($p < 0.01$). bFGF has the most pronounced stimulating influence upon the proliferation of the epithelium. By its kariokynetic activity bFGF surpasses EFR and several other growth factors.⁸

After orchiectomy an increase in the formation of IGF-1 ($p < 0.05$) was observed. This was promoted by the increase in the secretion of STH ($p < 0.05$). STH and IGF-1 manifest pronounced mitogen action.¹⁹

IGF-1 and a set of other peptides are the intermediary of the effect of estradiol. Their effect is manifested by the phosphorylation of the estrogenic receptors.^{17,29}

The receptor of IGF-1 is similar to the receptor of insulin, and therefore IGF-1 can connect with the receptors of insulin and activate them.¹⁹ The increase in the levels of IGF-1 ($p < 0.05$) and insulin ($p < 0.05$) in patients after orchiectomy is a compensatory answer to the development of insulin-resistance.

Insulin, along with IGF-1, raises the kariokynetic activity of the cells. From these positions it's possible to examine insulin-resistance as an instrument for the increase of the level of insulin, STH, IGF-1 and, accordingly, they're kariokynetic activity. The increase in the levels of the given indicators is characteristic of the stage of the promotion of tumor growth.^{17,30}

The increase in the aromatase activity and the

levels of the majority of growth factors after orchiectomy indicates that compensatory-adaptation reactions which develop when the level of testosterone goes down are directed towards an increase in the activity of the cells. Their expression is proportional to the degree of reduction of the testosterone level.

The given changes combine with the oppression of the formation of β TGF ($p < 0.05$) - a factor responsible for the consequential passage by the cell stages of differentiation and the start of apoptosis.^{8,17}

The cytokines IL-1 β and TNF α are produced by activated monocytes, macrophages and Th1.^{31,32} The initial increased levels of the given cytokines can be explained by the introduction of mechanisms of both specific and non-specific defenses into the anti-tumorous response. IL-1 β is a stimulator of the production of IL-2. The latter insures the proliferative phase of the immune response. The high levels of IL-1 β and TNF α were directed at the utilization of the formation of atypical cells.

After orchiectomy in the immune system, inhibiting effects predominate, and are manifested in the decrease in the synthesis of the cytokines IL-1 β and TNF α , which determine the formation of IL-2 and the formation of the T and NK cells.

TNF α , hydrolases (acid phosphatase, alkaline phosphatase, serin proteinases and esterases) components of the complement, and highly-active forms of oxygen and nitrogen determine to a significant degree the cytolytic (including anti-tumor) action of monocytes macrophages, neutrophils, and cytotoxic T cells and NK cells. After orchiectomy the initially-high levels of acid phosphatase, alkaline phosphatase and TNF α reliably decreased ($p < 0.01$; $p < 0.05$ and $p < 0.05$).

TNF α possesses expressed cytotoxicity, which is manifested in the destruction of tumorous cells. In addition to its regulatory and cytotoxic activity, TNF α also connects with specific membrane receptors, and in particular with Fas, which is the domain of cell death, and thus assists the launch of the program of apoptosis. Fas belong to superfamily of receptors TNF.^{31,32}

Acid phosphatase, which possesses cytolytic activity, is held in macrophages, in primary (aurophile) granules of neutrophils and in several other cells of the immune system.^{31,32}

Alkaline phosphatase also possesses cytolytic activity. It is held in secondary (specific) granules of

neutrophils and, equally to other highly-active substances and enzymes, is capable of causing death in tumorous cells.³¹

The formation of free radicles, peroxide, and other highly-active products is accompanied by a high use of glucose.^{31,32} The increased level of glucose, necessary for this process, is kept up owing to insulin resistance. After the orchiectomy the average value of the level of insulin reliably increased ($p < 0.05$), which points to the strengthening of the given compensatory mechanism.

In all patients before orchiectomy a high level of PSA was found. PSA is a serine proteinase - an enzyme, relating to the class of hydrolases. α_1 -antichemotripsine and α_2 -macroglobulin connect with PSA, converting it into an inactive form.^{17,33} Besides the epithelium of the prostate gland the serine proteinases are formed by a whole set of cells.

Granules of macrophages, neutrophils, cytotoxic T-cells and NK-cells also contain serine proteinases and esterases of the chemotripsin type. The regulation of the activity of the proteinases is done by their inhibitors, secreted by the macrophages: by α -anti-chemotripsin and α_2 -macroglobulin. Besides the manifestation of cytotoxicity, serine proteinases can cause in the cells-targets fragmentation of DNA and the start of the program of apoptosis, acting on the path of inner-cell signalization. Serine proteinases participate in the antibody-independent alternative path of activation of the complement.^{31,32}

Thus the increase of PSA, determined before orchiectomy in patients with prostate cancer, is not only the consequence of a secretion of the malignant cells of the epithelium of acinuses germinating the surrounding tissues entering into the blood stream, but also the expression of an overall compensatory activation of the cell immunity and of the complement system.

Ca⁺⁺ takes part in the activation of the complement, in the perphorin-dependent mechanism of interaction with the target cytotoxic T-cells and NK-cells.^{31,32} A month after the orchiectomy the average value of the level of Ca⁺⁺ rose ($p < 0.05$).

The presence of a single factor acting on patients (orchiectomy) allows one to connect all observed changes to the significant decrease in testosterone level. The reduction of the level of testosterone causes an increase in kariokinetic activity, the breach of the

regulation of the cell-cycle and the start of apoptosis, and (when the reduction of the level is significant) the decomposition of antitumor immunity. The given changes create the conditions for the development of a new tumor from low-differentiated androgen-independent epithelial cells, despite the dystrophic changes happening in the androgen-dependent cancer cells of the original tumor.

Taking into account the many-staged oncogenesis^{17,34} and the good, quick effect, it's possible to expect the androgen blockade to be very promising under the condition that it will be done in relatively short cycles, and that upon completion of the therapy the hormonal balance will be reduced. It's not possible to restore the chemical balance without the production of testosterone by the patient's own Leydig cells.⁷ For this reason only a pharmacological androgen blockade can be considered effective (in view of its reversibility).

REFERENCES

- Petrylak DP. Chemotherapy for the treatment of hormone-refractory prostate cancer. *Eur Urol Suppl* 2002; 2: 15-23.
- Stepanov VN, Shimanovskii NL. Androcure-preparation of the first line in palliative treatment of inoperable prostate cancer. *Moscow Med J* 2000; 8: 26-31.
- Pummer K. The role of urologist in the management of hormone-refractory prostate cancer. *Eur Urol Suppl* 2002; 2: 24-8.
- Pushkar DY. Radicle prostatectomy. Moscow: Medpress-inform, 2002: 7.
- Gray A, Feldman HA, McKinlay JB, Longcope C. Age, disease, and changing sex-hormone levels in middle-aged men: Results of the Massachusetts male aging study. *Clin Endocrinol* 1991; 73:1016-25.
- Vermeulen A, Kaufman JM. Androgens and cardiovascular disease in men and women. *The Aging Male* 1998; 1: 35-50.
- Pechersky AV, Semiglazov VF, Mazurov VI, Karpischenko AI, Mikhailichenko VV, Udintsev AV. Androgen administration in middle-aged and ageing men: effects of oral testosterone undecanoate on dihydrotestosterone, estradiol and prostate volume. *Int J Androl* 2002; 25: 119-25.
- Lopatkin NA. Guide to Urology. Moscow: Medicine, 1998(3): 374, 377-80, 385, 394-95, 504
- Sufi SB, Donaldson A, Jeffcoate SL. WHO Matched Reagent Programme Method Manual. 16th ed. London, UK, 1992:1992-3363.
- Whitley RJ, Meikle AW, Watts NB. Thyroid function. Tietz textbook of clinical chemistry. 2nd ed. Philadelphia: WB Saunders, 1994: 1698-739.
- Morales A, Bain J, Ruijs A, et al. Clinical practice guidelines for screening and monitoring male patients receiving testosterone supplementation therapy. *Int J Impot Res* 1996; 8: 95-97.
- Loran OB, Segal AS, Supryaga OM. Andriol in the treatment of secretion infertility and climacteric syndrome in men. *Urol Nephrol* 1999; 3: 41-4.
- Glantz SA. Primer of biostatistics. Moscow: Practica, 1999: 285-9.
- Kettail VM, Arki RA. Pathophysiology of the endocrinal system. St. Petersburg: Nevskii Dialect, 2001: 175, 232-3.
- Farber E. Cell proliferation as a major risk factor for cancer; a concept of doubtful validity. *Cancer Res* 1995; 55: 3759-62.
- Sporn MB. The war on cancer. *Lancet* 1996; 347: 1377-81.
- Bershtein LM. Hormonal carcinogenesis. St. Petersburg: Nauka, 2000: 10, 17, 31, 50, 51, 65, 69, 129.
- Russo J, Russo IH. Differentiation and breast cancer. *Medicina (B. Aires)*, 1997; 57: 81-91.
- Lavin N. Endocrinology. Moscow: Practica, 1999: 42, 43, 91, 93, 95, 102, 104, 346, 370-1, 373, 691, 877-98, 866, 966, 998-1004.
- Pankov AU. Prolactin. Big Medical Encyclopedia. Moscow: Soviet Encyclopedia, 1983 (21):134.
- Mendelson CR, Simpson ER. Regulation of estrogen biosynthesis by human adipose cells in vitro. *Molec and Cell Endocrinol* 1987; 52: 169-76.
- Ryde CM, Nicholls JE, Dowsett M. Steroid and growth factor modulation of aromatase activity in MCF7 and T47D breast carcinoma cell lines. *Cancer Res* 1992; 52: 1411-5.
- Bershtein LM. Extragonadal estrogen production - role in physiology and pathology. St. Petersburg: Nauka, 1998: 77, 86.
- Lueprasitsakul P, Longcope C. Aromatase activity of human adipose tissue stromal cells: effects of thyroid hormones and progestogens. *Proc Soc Exp Biol and Med* 1990; 194: 337-41.
- Jakob F, Homann D, Seufert J, Schneider D, Kohrle J. Expression and regulation of aromatase cytochrome P450 in THP1 human myeloid leukemia cells. *Molec and Cell Endocrinol* 1995; 110: 27-33.
- Burrows H, Horning E. Oestrogens and neoplasia. Springfield: Charles C Thomas Publ, 1952: 189.
- Zazzerov EG, Severin ES. Molecular mechanisms of oncogenesis of the prostate gland. *News of RAMN* 1998; 5: 29-35.
- Vasilev YM. The social behavior of normal cells and anti-social behavior of tumorous cells. *Soros Educational J* 1997; 4: 17-22.
- Lippman ME, Dickson RB. Mechanisms of normal and malignant epithelial growth regulation. *J Steroid Biochem* 1989; 34: 107-21.
- Yam D, Fink A, Mashiah A, BenHur E. Hyperinsulinemia in colon, stomach and breast cancer patients. *Cancer Lett*

- 1996;104: 129-32.
31. Yarilin AA. Foundations of immunology. Moscow: Medicine, 1999: 51-71, 111-44.
 32. Roitt I, Brostoff J, Male D. Immunology. Moscow: World, 2000: 63-4, 168-93.
 33. Loran OB, Pushkar DY, Frank GA. Prostate-specific antigene and the morphological characteristic of prostate cancer. Moscow: Medpress, 1999:13-4.
 34. Kopnin VR. Targets of action of oncogenes and tumorous supesores: the key to understanding the basic mechanisms of cancergenesis. Biochemistry 2000; 65: 5-33.