**Table 1. Clinical characteristics of the patients with adrenocortical cancer depending on the ENSAT disease stage**

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| **ENSAT stage** | **Number of patients, N (M:F)** | **Age, Ме (Q25–Q75), years** | **Number of patients with Cushing syndrome, N** | **Tumor size, (Q25–Q75), mm** |
| I | 8 (1:7) | 58 (45–62) | 2 | 45 (37–50) |
| II | 26 (6:20) | 47 (35–63) | 10 | 73 (66–99)  p = 0.007 |
| III | 14 (4:10) | 49 (38–63) | 7 | 118 (82–200)  р = 0.003 |
| IV | 11 (2:9) | 59 (43–62) | 9 | 130 (99–170)  p = 0.0001 |

F, female; M, male; p, significance of the difference between the patients with adrenocortical cancer stage II, III, and IV, compared to those in the stage I patients

**Table 2. Urine excretion of the main adrenocortical cancer biomarkers before surgery, depending on the disease stage, according to the results of gas chromatography-mass spectrometry, Ме (Q25–Q75), mcg/day**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Steroid** | **Control group (n = 28)** | **Adrenocortical cancer patients** | | |
| **Stage I (n = 8)** | **Stages II + III (n = 40)** | **Stage IV (n = 11)** |
| Ethiocholanolone | 677 (464–1261) | 480 (284–1054)  p = 0.43 | 3860\* (1633–6445)  p < 0.0001 | 6055\*\* (2056–10595)  p < 0.0001 |
| Androstendiol-17β | 99 (72–117) | 64 (55–80)  p = 0.27 | 1125\*\* (553–2292)  p < 0.0001 | 1923\*\* (723–3310)  р = 0.0002 |
| DHEA | 230 (122–330) | 129 (60–189)  p = 0.23 | 5317\*\* (1343–1300)  p < 0.0001 | 9231\*\* (5371–16299)  р = 0.0003 |
| 16α-ОН-DHEA | 185 (93–258) | 168 (141–1050)  p = 0.36 | 1487\*\* (658–3356)  p < 0.0001 | 6500\*\* (4704–18579)  р = 0.0002 |
| 16-oxo-androstendiol | 25 (15–45) | 65 (45–100)  р = 0.012 | 450\*\* (183–1055)  p < 0.0001 | 1617\*\* (558–2458)  p < 0.0001 |
| Androstenetriol | 189 (154–489) | 165 (88–1318) p = 0.79 | 1612\*\* (770–3270)  p < 0.0001 | 7206\*\* (4123–13047)  р = 0.0002 |
| 17-ОН-pregnanolone | 101 (54–232) | 128 (78–300) p = 0.48 | 1050\*\* (355–1676)  p < 0.0001 | 2140\*\* (1273–5172)  p < 0.0001 |
| Pregnanediol | 412 (251–644) | 1954 (555–3320)  p = 0.008 | 3356 (1781–6225)  p < 0.0001 | 9431\*\* (6320–15311)  p < 0.0001 |
| Pregnanetriol | 416 (370–584) | 943 (341–1158)  p = 0.49 | 2414\*\* (1259–4967)  p < 0.0001 | 7675\* (5079–23959)  p < 0.0001 |
| 11-oxo-pregnanetriol | 16 (11–21) | 39 (15–121)  p = 0.12 | 127 (60–598)  p < 0.0001 | 485 (59–788)  p = 0.0008 |
| Pregnenediol | 336 (200–564) | 315 (161–776)  p = 0.81 | 3084 (1562–5900)  p < 0.0001 | 6378\* (4345–8392)  p < 0.0001 |
| 3α,16,20-pregnenetriol | 150 (99–183) | 340 (223–1247)  p = 0.013 | 1069 (518–2778)  p < 0.0001 | 6755\*\* (2309–11255)  p < 0.0001 |
| 3α,17,20-pregnenetriol | 228 (111–317) | 382 (189–523)  p = 0.20 | 3081\*\* (1788–6444)  p < 0.0001 | 14657\* (3647–22000)  p < 0.0001 |
| Tetrahydro-11-deoxycortisol | 68 (25–99) | 126 (56–268)  p = 0.29 | 1081\*\* (397–2737)  p < 0.0001 | 3844\*\* (2920–4677)  p < 0.0001 |
| Tetrahydro-11-deoxycorticosterone | 12 (11–15) | 14 (10–19)  p = 0.14 | 203 (153–540)  p < 0.0001 | 613 (271–1140)  p = 0.001 |

DHEA, dehydroepiandrosterone; p, significance of the difference between the ACC patients and the control group

\* p < 0.001, \*\* р < 0.017, significance of the difference between the ACC patients with II + III and IV stages and the ACC stage I patients

**Table 3. Specific characteristics of steroid metabolomics in the patients with adrenocortical cancer (ACC) stages I to IV, according to the results of gas chromatography-mass spectrometry, Me (Q25–Q75)**

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| --- | --- | --- | --- | --- |
| **Parameter** | **Control group (n = 28)** | **Adrenocortical cancer patients** | | |
| **Stage I (n = 8)** | **Stages II + III (n = 40)** | **Stage IV (n = 11)** |
| The signs of 11β-hydroxysteroid dehydrogenase type 2 activity | | | | |
| THF/THE | 0.71 (0.59–0.81) | 0.99 (0.70–1.18)  p = 0.06 | 1.11 (0.67–1.49)  р = 0.005 | 0.83 (0.74–1.08)  p = 0.045 |
| (5β-THF+5α-THF)/THE | 0.34 (0.29–0.41) | 0.57 (0.42–0.78)  p = 0.001 | 0.77 (0.43–1.07)  p < 0.0001 | 0.56 (0.41–0.68)  p = 0.007 |
| ТНВ/ТНA | 1.6 (0.7–1.8) | 8.4 (3.9–23.9)  р = 0.003 | 4.7 (2.2–8.9)  р = 0.0005 | 3.1 (2.0–12.9)  р = 0.018 |
| The sign of 11β-hydroxylase activity | | | | |
| (5β-THF+5α-THF+ТНЕ) / tetrahydro-11-deoxycortisol | 96.3 (61.9–147.2) | 25.2 (17.1–93.4)  p = 0.15 | 5.8 (2.4–18.1)  p < 0.0001 | 1.9 (1.2–5.9)  p < 0.0001 |
| The signs of 21-hydroxylase activity | | | | |
| (5β-THF+5α-THF+THE) / 11-oxo-pregnanetriol | 157.9 (91.4–218.2) | 78.1 (42.4–137.9)  р = 0.088 | 31.0 (19.5–115.4)  p = 0.046 | 35.1 (20.6–180.7)  p = 0.005 |
| (5β-THF+5α-ТHF+THE) / pregnanetriol | 5.1 (3.7–6.6) | 5.9 (3.9–8.6)  р = 0.51 | 2.5 (1.1–4.1)  p = 0.0002 | 1.0 (0.2–3.6)  p = 0.0003 |
| (5β-THF+5α-THF+THE) / 17-ОН-pregnanolone | 20.1 (14.2–54.9) | 26.6 (16.5–51.3)  р = 0.97 | 7.2 (3.7–15.4)  p = 0.007 | 3.3 (1.7–16.5)  p = 0.001 |

Р, the significance of the difference between the ACC patients and the control group; THA, tetrahydro-11-dehydrocorticosterone; THB, tetrahydrocorticosterone; THE, tetrahydrocortisone; THF, tetrahydrocortisol