The effect of prolactin and bromocriptine on human peripheral immune status[1]

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ABSTRACT. The aim of this study was to determine the influence of elevated serum prolactin (PRL) levels on the peripheral lymphocyte subsets in patients with hyperprolactinemia. For this purpose we studied 20 hyperprolactinemic patient lymphocyte subsets by flow cytometry on their hyperprolactinemic state and after their serum prolactin concentration was normalized with bromocriptine (BC) alone or BC and surgery. We observed decreased absolute numbers and percentage of Natural Killer (p=0.0009 and 0.0001, respectively) and CD3/CD25 lymphocytes (p= 0.009 and 0.002) in hyperprolactinemic patients, compared to 8 sex- and age-matched normal controls. There was no correlation between PRL levels and CD16/56 and CD3/CD25 numbers (p=0.72 and 0.33, respectively). We did not find any significant difference in absolute numbers (p=0.95) and percentage (p=0.84) of B-lymphocytes of hyperprolactinemic patients, as compared with normal controls. We did not detect any increase in absolute cell numbers of CD16/CD56 (p=0.21) and CD3/CD25 (p=0.61) of BC-treated patients when compared to their hyperprolactinemic state. We demonstrated an increase in CD8-cells (p=0.0173) and a decrease in CD4/CD8 ratio (p=0.036) in hyperprolactinemic patients treated with BC. There was also an increase in the number of activated T-cells (CD3/HLA DR) in this group, compared to normal controls and the hyperprolactinemic state of the same patients (p=0.04). In conclusion, elevated PRL levels do not lead to an "overstimulation" of the B-cells, but deteriorate the cytotoxic function.

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INTRODUCTION

Involvement of the neuroendocrine system in immune regulation was first observed in hypophysectomized rats in 1930. Initial observations indicated that hypophysectomized rats present thymic atrophy and lymphopenia. This effect was reversed by the reintroduction of prolactin (PRL) to these rats (1). Thereafter, a number of studies have focused on the potential immunomodulatory roles of prolactin. Gerli *et al.* (2) demonstrated reduced Natural Killer (NK) cytotoxic activity in hyperprolactinemic patients, whereas Matera *et al.* (3) were unable to reproduce these findings. On the other hand, Cross *et al.* showed an increase in B-cell activity in hyperprolactinemia (4). In other studies, it was shown that the inhibition of T-cell function by hyperprolactinemia was more prominent than the stimulatory effect on B-cell functions in supraphysiologic PRL levels (5, 6). In animal models of autoimmune diseases, administration of dopaminergic agents suppressed the postpartum exacerbation of systemic lupus erythematosus (7), autoimmune uveitis (8) and collagen-induced arthritis (9).

Prolactin receptors have been shown on the membranes of white blood cells. The immunosuppressive agent cyclosporine A blockades these receptors and competes with PRL for binding to them. This may be one explanation of the immunosuppressive action of cyclosporin A (10).

The aim of this study was to determine the influence of elevated serum prolactin levels and the effect of a dopaminergic agent, bromocriptine (BC) on the peripheral immune status in patients with prolactinoma.

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PATIENTS AND METHODS

Patients selection

Twenty patients with hyperprolactinemia were admitted to the study after having given written informed consent. The subjects were considered as 3 different groups as follows:

- group 1: 20 patients with hyperprolactinemia;
- group 2: 8 age- and sex-matched normal controls;
- group 3: 14 patients of group 1, whose serum PRL levels were normalized with BC alone or with surgery and BC. In these patients all the tests were repeated at the end of first month of the treatment.

Study design

The first examination included clinical examination, and evaluation of PRL, SGOT, SGPT, TSH levels. Also a history of drug use which may produce hyperprolactinemia was obtained. PRL levels were measured in plasma samples collected every 20 min for three times.

Peripheral lymphocyte subsets were studied by flow cytometry in all subjects during hyperprolactinemia and after their serum PRL levels were normalized by BC or surgery and BC. Samples were immediately analyzed by cell counter (Cell Counter Microdiff. 18. Coulter Elect. Miami, U.S.A.) and by flow cytometer (Coulter EPICS XL MCL Coulter Elect. Miami, U.S.A.). Whole blood rather than Ficoll separated mononuclear cells was used for flow cytometric analysis. One hundred mµl of blood was pipetted to each tube, 10 mµl of isotype controls (DAKO inc.) and immunoflourescent antibodies (DAKO inc.) were added and incubated for 30 minutes at 4 C in the dark. Q-Prep (Coulter Elect. Miami, U.S.A.) was used for dye fixation and erythrocyte lysing. Two-color analysis (FITC/PE) was performed for CD2/CD25, CD3/HLA-DR, CD2/CD8, CD16/56, CD4/CD45RA, and CD4/CD45RO.

PRL levels were determined with ELISA (Merck, Darmstadt, Germany; normal: female<18 ng/ml,

Table 1 - *Prolactin (PRL) levels (ng/ml) and lymphocyte subsets distribution (absolute number, ab/mm^3) of patients in their hyperprolactinemic state (group 1), normal controls (group 2) and after bromocriptine (BC) therapy (group 3).*

	Group 1 no.=20	Group 2 no.=8	Group 3 no.=14
PRL	193.5 (62.48-628.00)	14.00 (11.25-19.25)	15.22 (3.40-42.92)
CD 16/56	157.70 (107.25-330.63)	566.00 (503-707.25)	132.82 (131.1-488.4)
CD19	303.35 (191.25- 1059.50)	313.00 (254.25-408.50)	573.55 (396.65- 1288.80)
CD3/25	12.70 (6.90-36.65)	98.00 (43.50-310.25)	21.00 (8.10-37.99)
CD3	1608.35 (971.18- 2091.60)	1466.50 (1302.50-1603.25)	1849.5 (1641.93- 2723.00)
CD4/45 RA	331.20 (255.53- 618.45)	464.50 (405.25-544.75)	562.80 (494.10- 654.94)
CD4/45 RO	342.00 (245.55- 761.30)	580.00 (500.25-643.5)	500.50 (423.67- 796.40)
CD4	943.45 (579.75- 1505.25)	1027.00 (912-1083.75)	1149.58 (1112.40- 1347.50)
CD8	552.60 (382.65- 803.03)	610.00 (536-756.25)	872.62 (696.59- 1088.50)
CD4/CD8	1.41 (1.18- 3.02)	1.63 (1.40-2.08)	1.29 (1.24-1.71)
CD3/HLA DR	122.60 (37.83- 589.40)	107.00 (88.75-347.25)	489.71 (283.52- 682.88)
Leucocyte	6750 (4835-8975)	6400 (5200-7900)	9300 (7600- 12800)
Lymphocyte	2450.00 (1500-3025)	2100.00 (1900-2100)	2700 (2336.90-3450.00)

Values are median and interquartile ranges.

male<17 ng/ml). Serial dilutions were performed, if necessary, to give an accurate evaluation of PRL levels.

Statistical analysis

Statistical analysis was done by Wilcoxon test in paired groups (group 1 and group 3), and by Mann-Whitney-U in non-paired groups. The Pearson product-moment correlation coefficient was used to evaluate the degree of correlation between all parameters. Significance was accepted if $p<0.05$. Calculations were made using SPSS software (SPSS, Inc., Evanston, IL).

RESULTS

Twenty subjects had hyperprolactinemia [14 women, 6 men, median age: 34 yr (range: 28-37)]. The cause of hyperprolactinemia was pituitary microadenoma in 13 patients, macroadenoma in 6, and empty sella in one patient. The median duration of the hyperprolactinemia was 24 months (range: 9-120 months) and the mean dose of BC required for normalization of PRL was 7.5±3.64 mg/day.

Prolactin levels and peripheral lymphocyte subsets distribution of patients is shown on Table 1.

We observed decreased absolute numbers and percentage of NK (CD16/56) (p=0.0009 and 0.0001, respectively) (Fig. 1) and decreased expression of interleukin-2 (IL-2) on T-lymphocytes (CD3/CD25) (p=0.009) (Fig. 2) in hyperprolactinemic patients, compared to 8 sex, and age-matched normal controls. There was no correlation between PRL levels and CD16/56 and CD3/25 cell numbers (p=0.72 and 0.33, respectively). We also could not determine any correlation between disease duration and CD16/56 and CD3/25 numbers (p=0.414 and p=0.48, respectively).

BC-treated patients (BC-patients), when compared to normal controls, had significantly higher total leukocytes (p=0.018), total lymphocytes counts (p=0.015), CD3 (p=0.04), CD3/HLA DR (p=0.01) and CD8 cells (p=0.01), but decreased CD16/56 cell numbers (p=0.002) and IL-2 expression on activated T-cells (p=0.002). There was also no increase in absolute cell numbers of CD16/56 (p=0.21) and CD3/CD25 (p=0.61) of BC-treated patients when compared to their hyperprolactinemic state. On the other hand, an increased expression of HLA-DR on T-lymphocytes (CD3/HLA DR) in this group was observed, when compared to normal controls and hyperprolactinemic state of the same patients before BC treatment (p=0.004 and 0.003 respectively). We demonstrated an increase in CD8-cells (p=0.017) and a decrease in CD4/CD8 ratio (p=0.036) (Fig. 3) in hyperprolactinemic patients, after treatment with BC.

DISCUSSION

An optimal amount of PRL must be present for maximal immune function. Either low or high levels of PRL may result in immunocompromise. Conflicting data regarding the effect of PRL on the immune system does exist, however. Mukherjee *et al.* (11) studied the *in vitro* effect of PRL

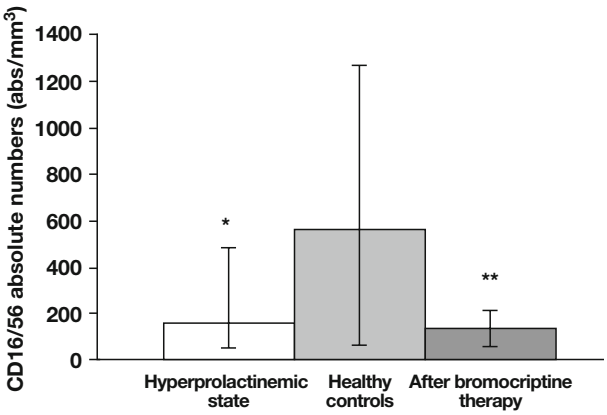

Fig. 1 - *Comparison of median absolute numbers of Natural Killer cells (CD16/56) between hyperprolactinemic patients (no.=20), normal controls (no.=8) and after bromocriptine therapy (no.=14). Results were expressed as the median and interquartile range. *p=0.0009; **p=0.002 (compared to normal controls by Mann-Whitney-U test).*

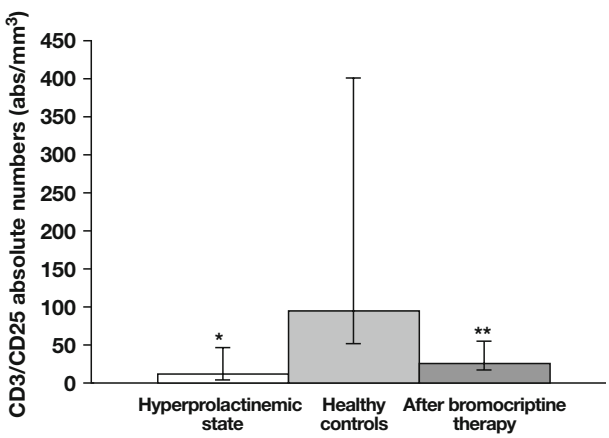

Fig. 2 - *A decrease was observed in expression of interleukin-2 (IL-2) on T-lymphocytes (CD3/CD25) in hyperprolactinemic patients, and after bromocriptine-therapy as compared with controls. Results were expressed as the median and interquartile ranges. *p=0.009; **p=0.002 (by Mann-Whitney-U test).*

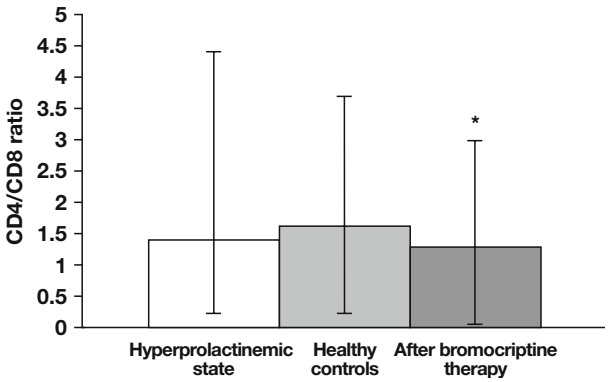

Fig. 3 - *CD4/CD8 ratio in hyperprolactinemic patients, after treatment with bromocriptine and in normal controls. The bars represent the median levels. 75th and 25th levels were also shown. *p=0.036 compared to CD4/CD8 ratio in normal controls.*

on splenocytes from ovariectomized rats and showed that PRL induced the formation of interleukin-2 cell surface receptors. On the other hand Koller *et al.* (12) investigated several immune parameters in nine patients with hyperprolactinemia. They did not demonstrate any changes in interleukin-2 receptor, CD45RO, and HLA-DR expression of CD4 or CD8 cells in the patients with prolactinoma, but they showed an increased CD4/CD8 ratio, which remained high after treatment and did not seem to correlate with serum prolactin concentrations. Vidaller *et al.* (13) studied four patients with tumoral hyperprolactinemia and showed that hyperprolactinemia was associated with a decrease in suppressor cell function, in the production of IL-2 and in the response to mitogens. In addition, PRL and hyperprolactinemia seem to suppress NK-cell activity. Gerli *et al.* (2) and Honorati *et al.* (14) have demonstrated reduced NK-cell activity in human hyperprolactinemic patients compared with BC-treated prolactinoma patients and healthy control subjects. Matera *et al.* (3) were unable to reproduce these findings, however.

Our data support the decreasing effect of PRL on the NK-cell numbers and the IL-2 receptor expression on T-cells. The lack of any correlation between PRL levels and these differences suggests that PRL may have suppressor effects on T-cells in high concentrations, however because of a non PRL-mediated effect, expected correlation may not be found. Although treatment with BC normalized PRL levels, NK-cell numbers and IL-2 expression were not changed. These findings suggest that BC also has an immune suppressive effect independent of its PRL lowering effect. Morikawa *et al.* (15) demonstrated that BC has a lowering effect on IL-2 production from T-cells, but BC poorly influenced the expression of IL-2 receptors. It was also remarkable that the BC causes an increase in total leukocyte and lymphocyte counts in our study due to increment in suppressor T-cells. BC also activates suppressor T-cells, which is characterized by increased CD3/HLA DR expression.

The absence of increased markers of B-cells such as CD19 (p=0.95) indicates that PRL in high concentration does not have any effect on B-cells.

The present results confirm the suppressor effect of hyperprolactinemia on NK-cell numbers, and the T-cell activation markers. On the other hand, the data demonstrates that supraphysiological PRL levels have no effect on B-cell numbers.

In conclusion, we suggest that hyperprolactinemia does not have any immune stimulatory effect on B-cells, but it has a suppressive effect on T- and NK-cells. These findings give insight on a suppressive role of prolactin on cytotoxic functions of immune system, rather than a stimulatory effect on it.

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