RELATIONSHIP BETWEEN VITAMIN D, CATHELICIN AND β2-DEFENSIN IN PATIENTS WITH DISORDERS OF EATING BEHAVIOR

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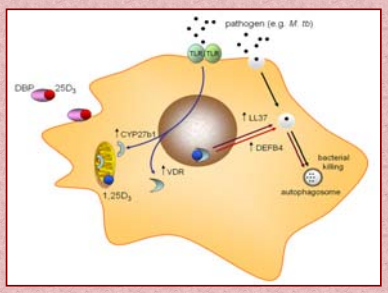
INTRODUCTION

Anorexia Nervosa is a serious disease characterized by abnormal behavior in a voluntary restriction of caloric intake and body image disturbance. Is not clearly established the incidence of infections in these patients, but many studies say that there are infrequently.

The antibacterial activity, bacteriostatic and bacteriolytic properties of vitamin D (25(OH)D and $1,25(OH)_2D$)) are well known. The $1,25(OH)_2D$, active metabolite, has receptors on many target cells, among which are included T lymphocytes, macrophages and dendritic cells**.** Therefore, Vitamin D with TLR (toll-like-receptor) and VDR, is considered as a modulator of the innate immune system, regulating positively the synthesis of antimicrobial peptides like ß2-Defensin and the Cathelicidin (hCAP-18, LL-37 or FALL-39) being the Cathelicidin the most important peptide in this way.

Cathelicidin, is a molecule composed of two parts: the catelin domain N-terminal highly conserved and a domain with antimicrobial activity at the C-terminal position that is highly variable. The differential activity of Cathelicidins is due to C-terminal domain .



OBJECTIVES

To establish the status of antimicrobial peptide, Cathelicidin and ß2-Defensin, and vitamin D (25(OH)D and $1,25(OH)_2D$) in patients with eating disorders that have been diagnosed by the criteria DSM-IV. Moreover, we analyzed the behavior of this peptides in these patients after administration of Vitamin D.

MATERIALS AND METHODS

Samples were collected (serum, EDTA plasma and urine) basal (before vitamin dose), 48 hours, and 2 and 6 weeks after treatment with 360.000 UI of Calcifediol (25(OH) vitamin D3), administered with a separation of 48 hours in 18 patients with eating disorders; the 6 weeks samples only were completed for 7 patients. In all the times we analyzed Ca++, PTH, 25(OH)D, 1,25 $(OH)_2D$ and a specific form LL-37, ß2-Defensin, and the human Vitamin D binding protein (VDBP).

The ionized calcium was measured in an autoanalyzer Ciba-Corning 634 (selective electrode) and PTH intact was determinated by chemiluminescence in an analyzer LIAISON® N-TACT® PTH (DiaSorin) with sensitivity 1 pg/mL and repeatability intra and interassay was respectively 2,6 and 5,8 %. The 25(OH)D was measured by specific immunoassays automated –chimioluminiscence- in a iSYS (IDS-iSYS). The sensivity was 5 pg/mL and intra and interassay precision were respectively 5 and 10%. The $1,25(OH)_2D$ was quantified by RIA (DiaSorin). Sensitivity was 2 pg/mL, and intra and interassay precision were respectively <10 and <15%. Human LL-37 was measured in plasma and urine by ELISA (Hycult biotech). Sensitivity was 0,14 ng/mL and, intra and interassay precision was <10 and 15%. For the quantitative determination of ß2-Defensin and VDBP were also determined in serum by ELISA (Alpha Diagnostic International® and Quantikine®, R&D respectively). Sensitivity was 5 pg/mL and 0,65 ng/mL respectively and intra and interassay precision were respectively <10 and <15% in both cases.

All data were included in the statistical package SPSS software (Statistical Package for Social Sciences, Chicago, IL, USA), for further processing. In the first time, the variables were submitted to the Kolmogorov-Smirnov test to evaluate the distribution, parametric or not, each of them. All data were expressed as the mean ± standard deviation; moreover, it also showed the median and range of non parametric variables.

Differences between times (basal, 48 hours, and 2 and 6 weeks) were performed by ANOVA, in the case of parametric variables, and Friedman test for nonparametric variables. If significantly differences were found, the variables were after analyzed with the Scheffé test (in the case of ANOVA) or with the Wilcoxon test with Bonferroni adjustment (in the case of Friedman test) to know between what specific times are the differences. To the correlations we used the "r" of Pearson test or the "s" of Spearman test in the same sense.

A **p** value less than 0.05 was considered significant. In the case of Friedman test use for non parametric variables a p value <0.025 was considered significantly due to Bonferroni adjustment.

RESULTS

2 M and 16 F were included in the study; 2 of them were diagnosed from Anorexia, 2 from Bulimia and 14 from EDNOS. BMI were respectively 17,7±0,2, 43,7±0,4 and 34,1±6,4. As you can see in the table, Vitamin D increased significantly at 48 hs and 15 days. ***Plasma Cathelicidin increase significantly at 15 days, but this response is observed only in the patients with deficit de Vitamin D (data not shown).***

Analyzing differences between vitamin D status nutritional (deficiency 25(OH)D <20 ng/ml). At the basal time patients with 25(OH)D<20 ng/ml have significant higher levels of urine ß2-Defensin, Med=193 (36-2583), than the patients with 25(OH)D >20 ng/ml Med=13 (5-235) (p=0,004).

On the other hand **analyzing the total results** plasma Cathelicidin correlated with serum ß2-Defensin (r=0,27 p=0,035) **(figure 1).** Serum ß2-Defensin correlates with urine Cathelcidin (r=0,34 p=0,008), with (Urine ß2-Defensin (r=0,53 p<0,001) and with age (r=-047 p=0,050) **(figure 2)**. Urine Cathelicidin correlated significantly with urine ß2-Defensin (r=0,43 p<0,001).

Analyzing **the correlations by times**, we observed that at the basal time it exists a negative correlation between urine ß2-Defensin and 25(OH)D (r=-0,57 p =0,014) and with $1,25(OH)_2D$ (r=-0,57 p=0,014). However, at 48 hs after vitamin D administration there is a positive correlation between ß2-Defensin and 25(OH)D (r=0,56 p=0,024) and with $1,25(OH)_2D$ (r=0,624 p=0,007) **(figure 3);** and at 15 days, it only exists a positive correlation between ß2-Defensin and 25(OH) D (r=0,55 p=0,023).

	Basal	48 hs	15 days	6 weeks	Anova o r Friedman test (p)
Edad (years)	31±10	-	-	-	-
Ca++ (mM)	1,22 ± 0,07	1,22 ± 0,04	1,22 ± 0,05	1,26 ± 0,05	0,404
Total CALCIUM (mg/dL)	9,1 ± 0,12	9,1 ± 0,33	9,2 ± 0,53	9,3 ± 0,46	0,812
PHOSPHORUS (mg/dL)	3,7 ± 0,59	3,8 ± 0,50	3,6 ± 0,62	3,6 ± 0,67	0,889
PTH (pg/mL)	90 ± 41	72 ± 48	60 ± 27	57 ± 20	0,098
25(OH)-VIT D (ng/mL)	18 ± 6	215 ± 72	224 ± 80	80 ± 25	<0,001 Basal – 48 hs p<0,001* Basal - 15 dias p<0,001*
$1,25(OH)_2$ –VIT D (pg/mL)	46 ± 18	110 ± 52	84 ± 29	46 ± 13	<0,001 Basal – 48 hs p<0,001* Basal - 15 dias p<0,017*
VDBP (µg/mL)	278 ± 95	279 ± 96	260 ± 69	255 ± 66	0,849
p CATHELICIDIN (ng/mL)	6,76 ± 8,10 3 (0,79 – 30,6)	5,95 ± 6,47 2,96 (0,86 – 23,25)	6,04 ± 6,55 3,30 (0,6 – 22,4)	3,99 ± 3,64 3,46 (0,38 – 9,60)	0,035 Basal – 15 days p=0,019**
u CATHELICIDIN (ng /mg creatinine)	0,27 ± 0,31 0,15 (0,07 – 1,36)	0,38 ± 0,71 0,15 (0,05 – 3,06)	0,31 ± 0,27 0,22 (0,04 – 0,92)	0,43 ± 0,48 0,33 (0,04 – 1,50)	0,834
s β2- DEFENSIN (pg/mL)	235 ± 190 186 (26 - 644)	240 ± 211 163 (40 – 747)	246 ± 188 163 (46 – 624)	145 ± 107 88 (24 – 310)	0,984
u β2- DEFENSIN (pg/mg creatinine)	272 ± 589 127 (5 – 2.583)	134 ± 124 96 (3 - 426)	346 ± 716 54 (6 – 1.109)	224 ± 408 41 (7 – 1.109)	0,901

Data are shown as meanSD in the case of parametric variables; we added median (range) in the case of non parametric variables. (*) Scheffé test or Wilcoxon test depending of parametric or non parametric variables. (**) This signification is the result of Friedman between the completed times. (p) plasma; (u) urine; (s) serum.

Figure 1

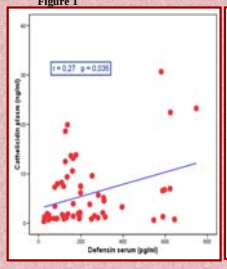


Figure 2

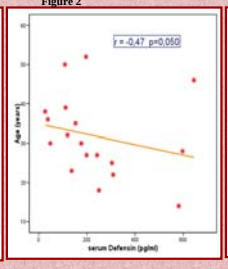
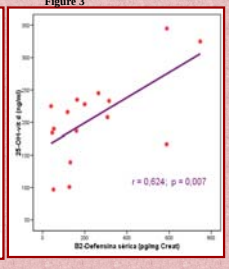


Figure 3



CONCLUSIONS

-After Vitamin D administration, there is a significant increase in 25(OH)D and $1,25(OH)_2D$ at 48 hs and 15 days. Moreover there is a significant increase in plasma Cathelicidin at 15 days.

-With this results, we demonstrated a negative correlation between Defensin with the age.

-In our study there are no differences in parameters analyzed between patients with sufficiency or not of Vitamin D except for 25(OH)D and PTH (data not shown), and for urine ß2-Defensin (with a negative correlation between 25(OH)D and serum ß2-Defensin) at the basal time. ***But, the significant increase in Cathelicidin after Vitamin D administration appears only in the patients with Vitamin D insufficient.***

-As you can see at the results, there is correlations between the two antimicrobial peptides analyzed, despite the small number of patients included at the moment.

-The change of sense of correlations between ß2-Defensin and 25(OH)D after Vitamin D administration is very interesting and more studies are necessary to known the physiology relationship between these two parameters.

References

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