Acta Poloniae Pharmaceutica – Drug Research, Vol. 72 No. 6 pp. 1279–1287, 2015 ISSN 0001-6837
Polish Pharmaceutical Society

CHANGES OF SALIVARY CORTISOL LEVEL AFTER VENLAFAXINE TREATMENT

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Abstract: Depression is one of the widespread diseases. Antidepressants used in the therapy of depression reduce the cortisol level but the impact of venlafaxine on the hormone secretion has not been explained so far. For this reason, the purpose of this study was to learn to what extent the venlafaxine therapy can affect the salivary cortisol level. Day-by-day cortisol quantitation in saliva of twenty depressed women was performed by HPLC. Statistical analysis and pattern recognition approach were used for interpretation of the results. Venlafaxine in mono- and combined therapy with sertraline, trazodone or mianserin has been found to have an impact on cortisol secretion as shown by the mean initial and final salivary cortisol levels, which were 76.01 ± 83.35 and 8.22 ± 7.15 ng/mL, respectively. The highest and lowest cortisol levels also indicate that fluctuation of the hormone secretion decreases during hospitalization and there are some different profiles of cortisol secretion as shown by the cluster and principal component analyses. The results have shown that venlafaxine used in combined therapy strongly reduced fluctuation of the cortisol level. For instance, for patients in which the fluctuation was found the mean hospitalization period was 42 days, whereas those treated with venlafaxine in monotherapy were hospitalized for almost 48 days. In conclusion, the combined therapy distinctly reduced the time of healing.

Keywords: cortisol, depressed women, pattern recognition approach, saliva, venlafaxine

Depression, one of the most common diseases around the world, can be caused by many factors. One of the theories claims depression as being due to long-lasting stress associated with increasing level of glucocorticosteroids (1). This is the effect of dysregulation of the hypothalamic pituitary adrenal (HPA) axis. Glucocorticosteroids have a profound effect on hippocampal cell proliferation, which is one of two brain regions where robust neurogenesis continues into adulthood. Another factor which has a crucial impact on the neurogenesis is stress, which reduces it. The nerve cells in hippocampal formation are the most sensitive to the deleterious effects of stress owing to enhanced release of glucocorticoids.

It has been found in the literature that in both depression and stress, an increase in cortisol secretion occurs (1). A crucial impact on the cortisol secretion have antidepressants, including selective serotonin reuptake inhibitors (SSRI) and serotonin and norepinephrine reuptake inhibitors (SNRI). Among SNRI, venlafaxine (VEN) is a commonly used drug in the therapy of depression. The mechanism of VEN action depends on the dosage used. A study of the impact of VEN on HPA axis by its serotoninergic effect and on cortisol secretion revealed that the hormone level in the healthy men blood increases proportionally to the dosage (2). The rise in cortisol secretion was also observed in saliva of healthy men and women, but at the beginning of night only (3). In the morning, its level fell down to the reference value. Furthermore, the low doses of VEN act as selective serotonin reuptake inhibitors and similarly to these drugs affect the cortisol secretion in healthy volunteers (4). The study has also shown that after 6 weeks of VEN therapy, in the majority of patients the cortisol level was lowered and the health was improved. At the same time, in the late responders group an insignificant reduction of the depression symptoms was observed, and additionally, the secretion of adrenocorticotropin hormone was still enhanced.

The differences were also observed in the impact of VEN therapy on cortisol secretion between responders and non-remitting patients (5).

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The results of cortisol quantification in saliva before therapy and repeatedly during the therapy showed that the hormone level was not lowered both in the depressed patients and the control group of healthy subjects. No significant impact of VEN on salivary cortisol was also shown by Hallam et al. (3). On the other hand, a significantly lower afternoon salivary cortisol was observed in the remitting than in non-remitting patients. Another theory on the increased level of cortisol during the depression hinges on variations in the activity of intracellular modulators of steroid activity, especially of two intracellular cortisol-deactivating enzymes. During the therapy with VEN, the levels of both enzymes returned to the level of control group (6).

Because VEN is a commonly used drug in the therapy of depression, it is interesting to monitor its influence on the changes of cortisol level during hospitalization of patients with major depressive disorder. This is crucial owing to the necessity of lowering the cortisol level during depression treatment. For this reason, the aim of this study was to learn to what extent the VEN therapy can affect variations in the salivary cortisol level. This purpose is justified by the fact that only a sparse information on the impact of VEN therapy on the cortisol secretion is available in the literature. In addition, these data are often controversial.

For quantitation of the cortisol level in the depressed patients a saliva has been chosen as diagnostic material (7, 8) to eliminate the influence of blood sampling stress on the hormone level. According to the literature data, a strong correlation between variations of the cortisol concentration in blood and saliva has been found to be of crucial significance for this study (9–11).

EXPERIMENTAL

Participants

The subjects with major depressive disorder (MDD) were recruited at the Hospital for Nervous and Mental Diseases in Starogard Gdański (Poland). All the participants were examined by a psychiatrist using the clinical interview and diagnosed according to the International Classification of Diseases (ICD-10) (12) criteria. All the subjects were informed in detail about the purpose of the study and gave their written consent to participate in it. The subjects were informed that they could discontinue the course at any time, if desired.

Some of the participants with MDD were excluded if their medical condition precluded administration of venlafaxine or if they had serious health problems, such as adrenal function disorders. Some other subjects were excluded because of the pregnancy and breastfeeding and if their participation in the course could be detrimental to their well-being. The participants were also excluded if they were unable to understand the nature of the study after discussion with a research nurse.

Finally, 20 women with MDD, 15 treated with VEN in monotherapy (MT) and 5 in combination therapy (CT) were included into the study. Table 1 summarizes the demographic and clinical characterization of the subjects, and a detailed information on the antidepressants and the doses used. The study had been approved by the ethical committee of the Medical University of Gdansk (Poland).

Quantitation of salivary cortisol

Salivary samples for quantitation of cortisol were collected from depressed women day-by-day during the whole hospitalization period, including the first day. The samples were collected into plastic tubes without any stimulation at 10 a.m. and frozen. The subjects were instructed to rinse their mouths with water, not to eat or drink 30 min before the samples have been collected.

For quantitation of salivary cortisol, a procedure of isolation and analysis has been developed by Dziurkowska and Wesolowski (13). The efficiency of cortisol extraction from saliva indicated that the recovery exceeded 94%. Validation of the HPLC method has shown that the coefficients of variation for the intra-day and inter-day studies varied from 1.1 to 6.5% and did not exceed 6.8%, respectively. The recovery fell in the range of 93.6 to 100.8%.

Statistical methods

All calculations were performed using a Statistica 7.1 (StatSoft, Kraków, Poland) software. The Wilcoxon test was used to study the impact of VEN therapy on salivary cortisol level in depressed women. With the aid of this test, the initial and final cortisol concentrations as well as the mean levels of the hormone during three periods of hospitalization, were compared. The influence of antidepressant therapy on the time of hospitalization was also checked. A one-way analysis of variance (ANOVA) test was used to study the correlation between the VEN dose and hospitalization period. To control the effect of VEN dosage on the mean concentration of salivary cortisol during three periods of hospitalization, ANOVA test for repeated measurements was used. The level of statistical significance was set at $p < 0.05$.

Two unsupervised pattern-recognition methods, cluster (CA) and principal component (PCA)

Table 1. Characteristics of the patients and the influence of venlafaxine in monotherapy and combined therapy on the cortisol level in saliva.

No.	Age of subjects (year)	Multiplicity of hospitalization	Dose used (mg)	Hospitalization period (days)	Cortisol concentration in saliva (ng/mL)		Highest and lowest cortisol concentrations in successive periods of hospitalization		
					initial	final	0–30%	31–60%	61–90%
1	51	4	75	90	173.62	0.25	0.25–173.62	0.25–40.00	0.25–18.75
2	52	1	75	28	158.75	11.25	2.50–158.75	2.50–22.50	2.75–31.25
3	47	2	75	31	41.25	8.75	1.62–50.00	3.75–36.25	2.50–32.50
4	57	1	75	63	15.00	9.87	1.25–32.50	1.25–62.50	1.75–13.50
5	47	2	CT	28	32.00	3.12	2.75–32.00	3.75–18.75	2.50–20.12
6	36	1	75	26	24.75	9.00	17.12–24.75	1.25–13.12	9.00–10.00
7	29	1	75	34	372.50	11.87	32.00–372.50	7.50–17.50	4.75–11.87
8	53	2	75	40	91.50	20.00	5.50–91.50	2.50–36.25	3.12–20.00
9	53	1	150	50	75.00	5.25	3.87–75.00	2.50–32.50	2.62–57.50
10	44	2	CT	46	41.00	3.62	2.50–41.00	1.50–10.00	1.25–23.75
11	44	2	75	87	40.00	9.25	1.75–40.00	2.50–30.62	2.62–32.50
12	47	3	CT	39	5.62	1.50	4.75–12.12	2.75–12.12	1.50–10.12
13	59	1	CT	52	95.00	11.37	4.87–95.00	4.75–32.50	2.50–11.37
14	26	1	75	68	90.00	7.25	3.25–90.00	2.87–15.00	3.25–11.50
15	47	1	150	14	70.00	3.25	27.50–70.00	6.00–58.75	3.25–6.00
16	48	2	75	18	84.75	3.50	5.62–84.75	4.25–6.50	3.50–7.50
17	52	1	75	36	15.00	2.62	1.50–15.00	1.25–7.75	1.37–7.25
18	46	4	75	68	32.75	3.75	4.87–32.75	1.37–4.25	1.25–11.00
19	31	3	75	66	40.00	31.25	1.25–40.00	1.50–13.75	1.50–31.25
20	56	6	CT	45	21.75	7.62	4.50–38.50	3.12–15.00	2.81–19.75
$\bar{x}$	46.25	2.05	–	46.45	76.01	8.22	6.46–78.49	2.85–24.28	2.70–19.72
SD	9.22	1.36	–	21.44	83.35	7.15	8.73–81.79	1.77–16.53	1.79–12.50

CT – combination therapy (VEN in polypragmasy with sertraline 50 mg, trazodone 300 mg or mianserin 60 mg)

analyses, were used for data interpretation (14, 15). A matrix of the data with dimensions $n \times p$ was used, where n is the number of rows, including women with MDD, and p is the number of variables describing the studied subjects, such as age, hospitalization time and its multiplicity, initial and final cortisol level, the highest and the lowest cortisol levels and the difference between these values, mean concentration, median, stabilization of cortisol secretion at 8, 10 and 15 ng/mL during the whole hospitalization period and at different hospitalization phases. The matrix was 20×22 in dimension. In this study, a strategy without rotation of factors gave the best results in PCA, while in CA Ward's hierarchical agglomeration with Euclidean distance measure ensured the most interpretable results.

RESULTS AND DISCUSSION

Analysis of the data compiled in Table 1 has shown that 20 women aged 46 ± 9, spent in the hospital about 46 ± 21 days. The patients treated with VEN in monotherapy were hospitalized for almost 48 days and in the case of women under combination therapy with VEN the mean hospitalization period was 42 days. The level of salivary cortisol was quantified every morning. The results have shown that the mean initial and final values were 76.01 ± 83.35 and 8.22 ± 7.15 ng/mL, respectively.

Statistical analysis of the data listed in Table 1 shows that the initial cortisol levels differ significantly from the final hormone levels ($Z = 3.9199$, $p = 0.0001$), but the dose of VEN had no influence either on the initial or final concentrations of the hormone. Moreover, statistically significant differences were found ($Z = 3.5893$, $p = 0.0003$) between the mean cortisol concentrations measured during 30% of hospitalization period and those determined during 60% of the period. In contrast to this, there were no significant differences in the mean cortisol concentrations between the values measured during 60% and 90% of hospitalization periods ($Z = 0.5973$, $p = 0.5503$). Furthermore, the dose of VEN ($F = 0.0919$, $p = 0.9124$) and time of hospitalization ($F = 1.9598$, $p = 0.2715$) did not have an impact on the mean concentrations of salivary cortisol during three periods of hospitalization. On the other hand, statistically significant differences in the time of hospitalization were found between mono- and combined-therapy ($Z = 3.9199$, $p = 0.0001$).

The results of statistical analysis show that the VEN therapy has an impact on the cortisol content in saliva of depressed women (statistically significant differences between the initial and final cortisol levels, and between the mean hormone levels during the first and second hospitalization periods). These results are compatible with the literature data (4, 6).

Fluctuation of the salivary cortisol level

Day-by-day quantitation of the cortisol content in saliva of the subjects shows that a decrease in hormone level can take place in different ways. Detailed inspection of these data revealed that there are four different profiles of salivary cortisol changes. The first one presented in Fig. 1A relates to the patients whose organism responded to the treatment. The cortisol secretion decreased very quickly attaining the reference values. In saliva, the reference values for a healthy person are between 1 and 8 ng/mL (16). This level did not increase up to the end of hospitalization. The differences between cortisol concentrations in saliva as determined day-by-day surpassed few nanograms per milliliter. Hospitalization period of those patients did not exceed 25 days.

When the cortisol concentration decreased slower and the hospitalization exceeded 20 days, there were a few types of the cortisol secretion profiles. In the case of higher than 60 ng/mL initial cortisol levels, secretion of the hormone dropped in the first days of treatment achieving the reference values (Fig. 1B). In the next few days, the cortisol secretion increased and the amplitude of its concentration in saliva was larger than that during the stabilization period.

Another profile of the cortisol secretion is shown in Fig. 1C. When initial cortisol concentration was about 40 ng/mL, its secretion dropped very quickly attaining the reference values. In the case of patients staying at the hospital for more than 30 days, an increase in the hormone secretion occurred at about 30th day of hospitalization. After a few next days, the secretion declined and to the end of hospitalization the amplitude of cortisol level fell in the range of several or several dozen nanograms per milliliter of saliva. This revealed that the fluctuation of the cortisol secretion had a distinct impact on hospitalization time. This refers especially to patients with a large amplitude of cortisol secretion, typically several dozen nanograms per milliliter of saliva. In that case, the hospitalization period was longer than 30 days for the majority of depressed women.

About 20% of the studied patients treated with VEN in MT did not respond to the treatment. Therefore, five depressed women were treated in polypragmasy with VEN and sertraline, trazodone or mianserin. The mean hospitalization period was

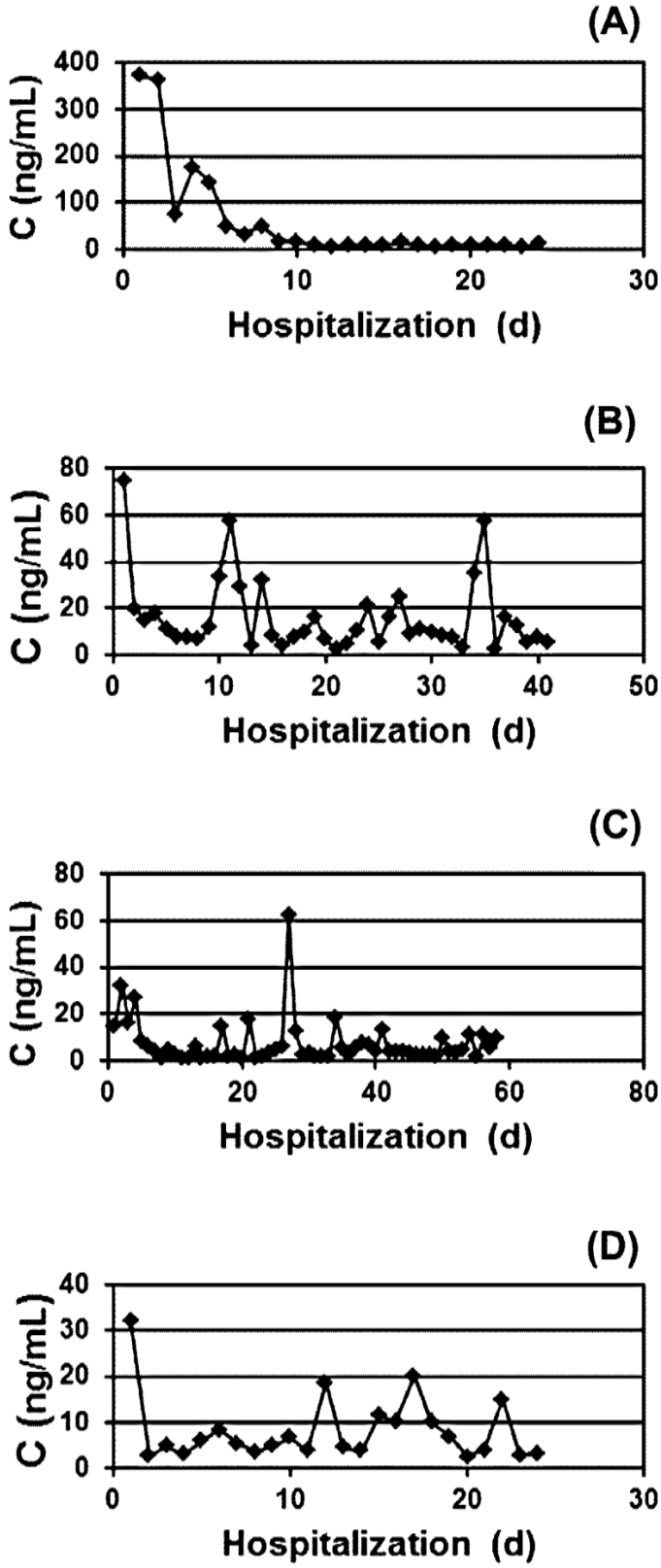

Figure 1. Profiles of cortisol secretion in women with major depressive disorder. Patients treated with venlafaxine: (A), (B), (C) – in monotherapy, (D) – in polypragmasy with sertraline, trazodone or mianserin

42 ± 9 days and was shorter than that for patients treated with VEN in monotherapy. Furthermore, differences between the salivary cortisol quantified day-by-day were lower, as is shown in Fig. 1D. This also refers to patients with a high initial level of cortisol, of about 100 ng/mL. The hormone concentration decreased in a few days and did not exceed 20 ng/mL up to the end of hospitalization. The second rise of the cortisol level at about 30th day of hospitalization did not occur as compared to patients treated with VEN in monotherapy.

To sum up, the impact of VEN therapy on cortisol secretion is diverse and depends on the state of health of the patient and also remitting of the treatment (5). This study supports these observations. In the case of a patient hospitalized for more than 30 days, a significant impact on cortisol level quantified day-by-day was noticed. Furthermore, the profiles of cortisol secretion revealed an increase in the hormone secretion at about 30th day of hospitalization. This rise of the cortisol secretion was not observed in the group of patients treated with VEN

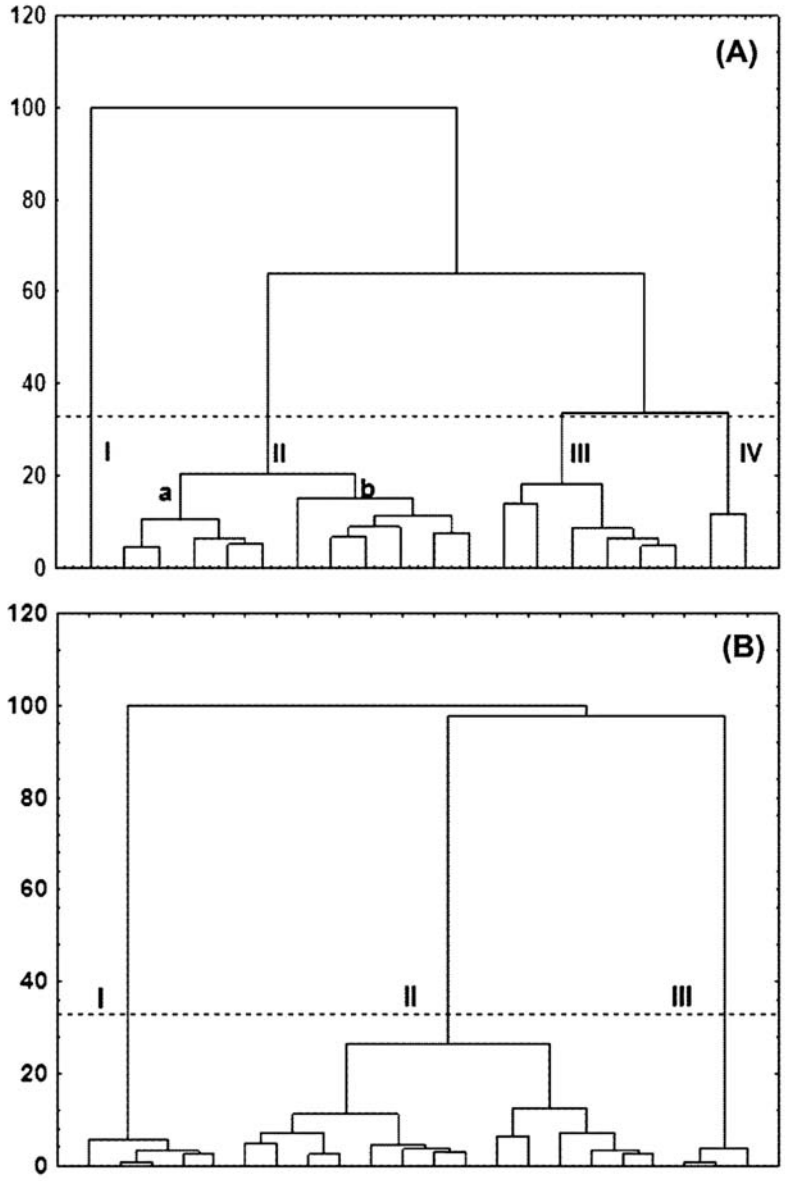

Figure 2. CA dendrograms illustrating the clustering of 20 subjects (A) and 22 variables (B), based on the data of Table 1

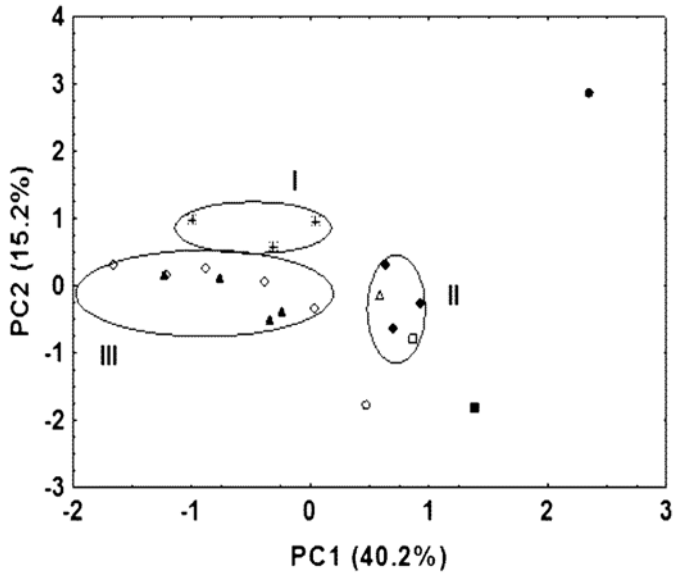

Figure 3. PCA score plot showing the grouping of 20 depressed women. Patients fall in Figure 2A in cluster I (*), cluster II (◇), and cluster III (◆). The youngest patient (●). Patients with high cortisol fluctuation (○), the shortest period of hospitalization (■), treated with 150 mg of VEN (□), treated in CT with mianserin or trazodone(▲), and treated in CT with sertraline (Δ)

in CT. This can be explained in terms of a relatively weak effect of VEN on the glucocorticoid receptor (GR) function to cause that the controlling of cortisol secretion is not strong. It also depends on the dosage of the drug (17). This study has shown that VEN used in combined therapy (CT) with other antidepressants, such as trazodone or mianserin, enhance the influence on GR. In the case of treatment with VEN and sertraline, which also affect rather weakly GR function, the fluctuation of cortisol level was larger. It is in a good agreement with the literature data on the cortisol secretion during SSRI therapy of depression (18).

Multivariate analysis of the results

Based on the day-by-day fluctuations of the cortisol concentration it was found that there are some different profiles of cortisol secretion as illustrated in Figure 1. To learn whether or not particular profiles differed widely in salivary cortisol level, a pattern recognition approach based on CA and PCA was used (14, 15).

Cluster analysis enables presentation in the form of a dendrogram, a way of grouping patients into particular clusters, with samples belonging to one cluster characterized by high similarity, and simultaneously, differing to the maximum extent from the other samples. As shown in Fig. 2A, twenty subjects with MDD were grouped into four clusters determined at 1/3 of the maximum distance using a Sneath's index criterion. A detailed analysis of the subjects found in these clusters revealed that variations in the amplitude of cortisol level in saliva had a vital impact on their classification. In the first cluster falls one patient only with a profile of salivary cortisol presented in Figure 1A. This subject is characterized by the highest initial concentration of cortisol in saliva (above 370 ng/mL) which decreased in the next few days to attain the reference value. As this patient differs strongly from the others, clusters II–IV are situated at a maximum distance from this one.

Clusters III and IV are the next ones which differ mostly from each other. Cluster III is formed by five patients treated with VEN in MT with profiles of cortisol changes shown in Figure 1B and one treated with VEN in CT. Almost all of them were hospitalized for the first time. In all cases the initial cortisol level was about 80 ng/mL. Those patients are characterized by enhanced fluctuation of the hormone secretion, which did not exceed 10 ng/mL. In the patient treated with VEN in polypragmasy with sertraline, the amplitude of cortisol level was substantial only at the beginning of hospitalization. On the other hand, cluster IV grouped 2 patients with a high initial cortisol level (above 150 ng/mL) which after a few days declined similarly as in the case of the patient from cluster I. The cortisol levels quanti-

fied day-by-day fell in the range of several nanograms per milliliter of saliva, especially at the end of hospitalization. Cluster IV encompassed two women, one with the longest period of hospitalization, 90 days.

Most of the patients studied (11) are located in cluster II, which can be divided into two subclusters, a and b, below 20% of the maximum distance. In this cluster are located four of the five subjects treated with VEN in polypragmasy. Three of them are in subcluster IIa, which encompasses patients with low amplitude in the hormone level, a few nanograms per milliliter during the whole period of hospitalization. The highest level of cortisol achieved in saliva of those patients did not exceed 20 ng/mL with a profile of cortisol secretion shown in Figure 1D. Subcluster IIb is formed by subjects with an enhanced fluctuation of cortisol secretion during the whole hospitalization period. In this case, the amplitude of cortisol level determined day-by-day was large, more than several dozen nanograms per milliliter. This subcluster encompasses patients with cortisol profile represented in Figure 1C, with initial cortisol level of about 40 ng/mL. The majority of these subjects were hospitalized because of the depression more than once.

The relation between 22 variables characterizing the subjects with MDD is shown in Figure 2B. CA revealed a strong correlation among multiplicity of hospitalization, highest and final cortisol levels, mean and median (cluster I). In this way CA connects multiplicity of hospitalization with salivary cortisol level. Another one was formed by variables relates to stabilization of the cortisol secretion at different levels during the whole hospitalization period and at different phase of the treatment, and also between hospitalization period and age of the patients. Both the initial and the lowest cortisol levels as well as the difference between the highest and the lowest levels are grouped in cluster III.

Another multivariate approach, PCA, enables interpretation of the data by reduction of dimensionality and adding clarity to presentation of the results. Because two first principal components (PCs), PC1 and PC2, explained more than 55% of the variability, a PCA score plot could be presented in the form of a two-dimensional plane (Fig. 3). In this case, the patients are grouped in three clusters. Cluster I is created by subjects with a high or very high level of initial cortisol concentration (between 84 and 173 ng/mL), but in this group the stabilization of cortisol secretion at the reference values has been achieved. Women with high initial cortisol concentration (about 100 ng/mL) and patients with high fluctuation of the cortisol during the whole hospitalization period are located in cluster II. The cortisol secretion profile for women located in this cluster is shown in Figure 1B and for one subject in Figure 1D. The woman marked with a white square was treated with 150 mg of VEN, whereas that marked with a white triangle was treated in polypragmasy with sertraline. Cluster III is created by patients with a cortisol secretion profile shown in Figure 1C. The initial cortisol concentration did not exceed 40 ng/mL. There are four out of five subject treated with VEN in polypragmasy (marked with a black triangle) with salivary cortisol profile shown in Figure 1D. The results are similar to those obtained by CA as presented in Figure 2A, cluster II.

It should also be mentioned that similarly as in CA (Fig. 2A, cluster I), an outstanding patient marked with a dot is located separately in the right corner of the PCA plot. Furthermore, the next two subjects are also located separately. The first marked with a black square is characterized by a very short hospitalization period and decreasing cortisol secretion during the first days of treatment. The other (white point) demonstrated the high fluctuation of hormone secretion.

The most significant impact on classification of the subject according to the PC1 axis had the stabilization of cortisol secretion at different levels during the whole hospitalization period and after elapsing of 30% of the period at the levels of 8 and 10 ng/mL of cortisol. The mean concentration was important as well. According to PC2 axis, the initial and the lowest cortisol levels, and also stabilization of cortisol secretion at 15 ng/mL after 30 and 60% of the hospitalization period were the most important factors for classification of the patients.

CONCLUSIONS

Based on the results obtained in this study, it can be stated that the VEN used in the depressed women therapy in MT and CT with sertraline, trazodone or mianserin has an impact on the cortisol secretion, the secretion has been suppressed. This can be supported by concentration of the hormone at the end of hospitalization, i.e., by the mean initial and final salivary cortisol levels which were 76.01 ± 83.35 and 8.22 ± 7.15 ng/mL, respectively. Furthermore, based on the highest and the lowest cortisol levels during different periods of hospitalization, it can generally be concluded that fluctuation of the hormone secretion decreases during hospitalization. Thus, it can be stated that there are some different profiles of cortisol secretion as shown in Figure 1.

These findings also demonstrate that VEN used in CT significantly reduced the fluctuation of cortisol concentration as quantified day-by-day during the whole hospitalization period. For instance, for the women under CT therapy with VEN the mean hospitalization period was 42 days, whereas those treated with VEN in MT were hospitalized for almost 48 days. In conclusion, the combined therapy distinctly reduces the time of cure.

REFERENCES

1. Jacobs B.L., van Praag H., Gage F.H.: Mol. Psychiatry 5, 262 (2000).
2. Daffner-Bugia C., Laakmann G., Voderholzer U., Haag C., Baghai T. et al.: Hum. Psychopharmacol. Clin. Exp. 11, 1 (1996).
3. Hallam K.T., Begg D.P., Olver J.S., Norman T.R.: Hum. Psychopharmacol. Clin. Exp. 23, 129 (2008).
4. Araya A.V., Rojas P., Fritsch R., Rojas R., Herrera L. et al.: Endocrine 30, 289 (2006).
5. Scharnholz B., Weber-Hamann B., Lederbogen F., Schilling C., Gilles M. et al.: Psychiatry Res. 177, 109 (2010).
6. Römer B., Lewicka S., Kopf D., Lederbogen F., Hamann B. et al.: Neuroendocrinology 90, 301 (2009).
7. Douglas J.-L., Harmer C.-J.: Eur. Psychiatry 26, 479 (2011).
8. Pfaffe T., Cooper-White J., Beyerlein P., Kostner K., Punyadeera C.: Clin. Chem. 57, 675 (2011).
9. Baghai T.C., Schule C., Zwanzger P., Minov C., Holme C. et al.: Psychoneuroendocrinology 27, 385 (2002).
10. Bhagwagar Z., Hafizi S., Cowen P.J.: Psychopharmacology 163, 118 (2002).
11. Lilliecreutz C., Theodorsson E., Sydsjö G., Josefsson A.: Arch. Women's Ment. Health 14, 405 (2011).
12. WHO 2013. Available at: http://apps.who.int/classifications/apps/icd/icd10online/. Accessed June 04, 2014.
13. Dziurkowska E., Wesolowski M.: Chromatographia 70, 769 (2009).
14. Hill T., Lewicki P.: Statistics, methods and applications: a comprehensive reference for science, industry, and data mining. StatSoft, Tulsa 2006.
15. Otto M.: Chemometrics: statistics and computer application in analytical chemistry, 2nd edn.,. Wiley-VCH, Weinheim 2007.
16. Jönsson B.A.G., Malmberg B., Amilon A., Garde A.H., Orbaek P.: J. Chromatogr. B 784, 63 (2003).
17. Augustyn M., Otczyk M., Budziszewska B., Jagła G., Nowak W. et al.: Pharmacol. Rep. 57, 766 (2005).
18. Dziurkowska E., Wesolowski M., Dziurkowski M.: Arch. Women's Ment. Health 16, 139 (2013).

Received: 6. 10. 3014