ORIGINAL ARTICLE

Evaluating Serum Proteome in Women with Obsessive-Compulsive Disorder/Bipolar Disorder Compared to Pure Obsessive- Compulsive Disorder Subjects and Healthy Controls

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Abstract

Objectives

The present study aimed to evaluate the serum proteome of women with obsessive-compulsive disorder (OCD)/bipolar disorder (BP) compared to pure OCD subjects and healthy controls.

Materials & Methods

Serum proteome of women with OCD/BP, pure OCD individuals, and healthy controls were subjected to 2DE-based proteomics accompanied with MALDI-TOF-TOF mass spectrometry. Further evaluation of the identified protein spots with the significance of $p<0.05$ and fold≥1.5 was done by applying protein interaction mapping via Cytoscape v. 5.3.1 and its plugins.

Results

The results indicate that vitamin D binding protein (GC) and haptoglobin spots (HP) significantly changed expression in OCD and OCD/BP with different expression patterns. These identified spots may contribute to OCD/BP and act as differentially recognized biomarkers comparing pure OCD and OCD/BP.

Conclusion

The Findings imply that these proteins in the serum of the patients could be potential distinguishable biomarkers in clinical usage after related validation experiments. Therefore, this study provides a preliminary evaluation to understand OCD/BP proteome behavior better.

Keywords: Obsessive Compulsive Disorder; Bipolar Disorder; Comorbidity; Proteomics; 2D Electrophoresis; Serum Profiling

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Introduction

Comorbidities in mental disorders are frequent. The obsessive-compulsive disorder (OCD)/bipolar disorder (BP) comorbidity is one of the most known challenges for treatment approaches (1). The pooled prevalence rates for OCD in BP and BP in OCD are 17-18%, as reported by a recent Meta-analysis study (2). In this sense, many studies have been conducted to understand this co-occurrence's clinical properties and treatment outcomes (2, 3). However, more research is required to understand the disorder better and choose a better treatment (2). For instance, serotonin reuptake inhibitors (SSRIs) are the first-line medication applied for OCD patients. However, the treatment options may be more complicated when it manifests with other conditions.

The manifestations include mood instability, worsened OCD symptoms, and drug-induced (4, 5). In this regard, approaches that are more effective worth pursuing. On the other hand, molecular investigations of OCD/BP are minimal. Most of this area's research focuses on genetics and genomic concepts of this mental disorder (6). A complex interaction between expressed genes and the surrounding environment exists in psychiatric disorders. These phenomena concluded with diverse abnormal phenotypes known as disorder spectrum. The clinical characteristics of OCD/BP are related to how these interactions occur (7). However, no research has been conducted on proteomics. Proteomics simultaneously detects abnormally expressed proteins in one experiment for a specific disease (8). Proteome analysis can provide essential information related to functional levels. In other words, organism functions are mediated through complex protein interactions. The dysregulation of these elements can lead to perturbation of specific interactions and interrupted biological processes. Therefore, at this scale, it is possible to recognize the disorder's underlying mechanisms (9). In addition, proteome profiling can assist monitoring changes in protein levels as the symptoms of the disease change, disease development, and treatment responses (10). One useful source for the mentioned goals is serum proteome profiling. By studying serum, it is possible to evaluate disease-correlated proteins (7). Consequently, the serum proteome of women patients with OCD/BP comorbidity is studied and compared with OCD patients to better understand the associated underlying mechanisms.

Materials & Methods

The proteomic evaluation was conducted in the Proteomics Research Center, Shahid Beheshti University of Medical Sciences. The samples were obtained from patients (2017) referred to Taleghani Hospital, Tehran, Iran. The samples included twenty healthy women without a history of mental disorders demography matched with 12 moderate OCD and 10 OCD/BP patients without any background of taking treatments. The diagnostic test was based on DSM-V criteria applied by two experts. The selected patients had no previous history of drug application, were aged between twenty and thirty years old, and had no other comorbidities.

Sample Preparation

Blood samples were collected from the healthy and patient participants using a venipuncture route and needle with a gauge of 2°C. Clotting was at room temperature for 30 min, and then the serum samples were entirely separated by centrifuging two times at 4ºC with 2000 g and 10 min duration.

Proteomic Analysis

The applied materials were from GE HealthCare Life Sciences (http://www.gelifesciences.com) and SERVA Company (http://www.serva.de). The 2-DE Clean-Up Kit (GE Healthcare) and 2-DE Quant Kit (GE Healthcare) were applied for protein extraction and quantifications. Before the first step of 2DE, IPG strips were subjected to passive rehydration for eight hours. The Isoelectric focusing (IEF) separates proteins based on their pI for 7.5 hours at 20 °C by applying Bio-Rad PROTEAN IEF Cell and 11 cm nonlinear IPG with a pH range of 4-7 according to Bio-Rad Protocol. Strips were subjected to an equilibration (Serva kit) for 30 min at room temperature between the first and second dimensions. The second dimension was done by the use of HPE FlatTop Tower (horizontal electrophoresis) using 2D HPE™ Double-Gel 12.5 % Kit (Serva Company) based on molecular weight (MW) for about 3.5 hours. After the 2DE steps, the gel samples were stained by applying SERVA HPE™Coomassie® Staining Kit according to the protocol and then scanned using a calibrated GS-800 densitometer (Bio-Rad) scanner (11). Analysis of protein spots was done using the Progenesis SameSpots Software as an image analyzer. The three samples were analyzed and compared in this way. Spots with a 1.5-fold value and a significance of $p \leq 0.05$ were considered significantly differentially expressed proteins. All the 2DE procedures were replicated three times. The candidate protein spots were identified using MALDI-TOF-TOF MS and MASCOT (http://www.matrixscience.com).

Interaction Analysis

The identified proteins were further investigated for interaction annotation, in which proteins interact with related compounds by Cytoscape software. The confidence score cutoff was 0.4, and 10 neighbors were considered.

Results

The proteomic analysis of the OCD/BP patients' serum showed differentially expressed proteins in this comorbidity compared to OCD samples and healthy controls. Vitamin D binding protein (GC) and haptoglobin spots (HP) are the identified statistically significant changed proteins in expression levels. The information related to expression trend and its statistical aspects is shown in Table 1, Figure 1, and Figure 2.

Interaction annotation of the identified differentially expressed proteins in OCD samples compared with OCD/BP samples. (See Figure 3)

Table1. The significant expression changes of GC and HP proteins in three conditions (Control, OCD, and OCD/BP)

#	Anova (p)	Fold C-O	Fold C-OB	Fold O-OB	Notes	pI	MW	Average Normalised Volumes		
								Control	OCD	OCD/BP
1		4.1	1	4.3	GC	5.41	157	514	2107	493
2	4.490e-009	1.7	3	1.7	HP	6.30	45	12810	7512	4331

The expression pattern of vitamin D binding protein (GC) in the three evaluated conditions are indicated in figure1.

Position (279, 204)
Notes
🟩 Fold>=1.5

🟥 p<=0.05

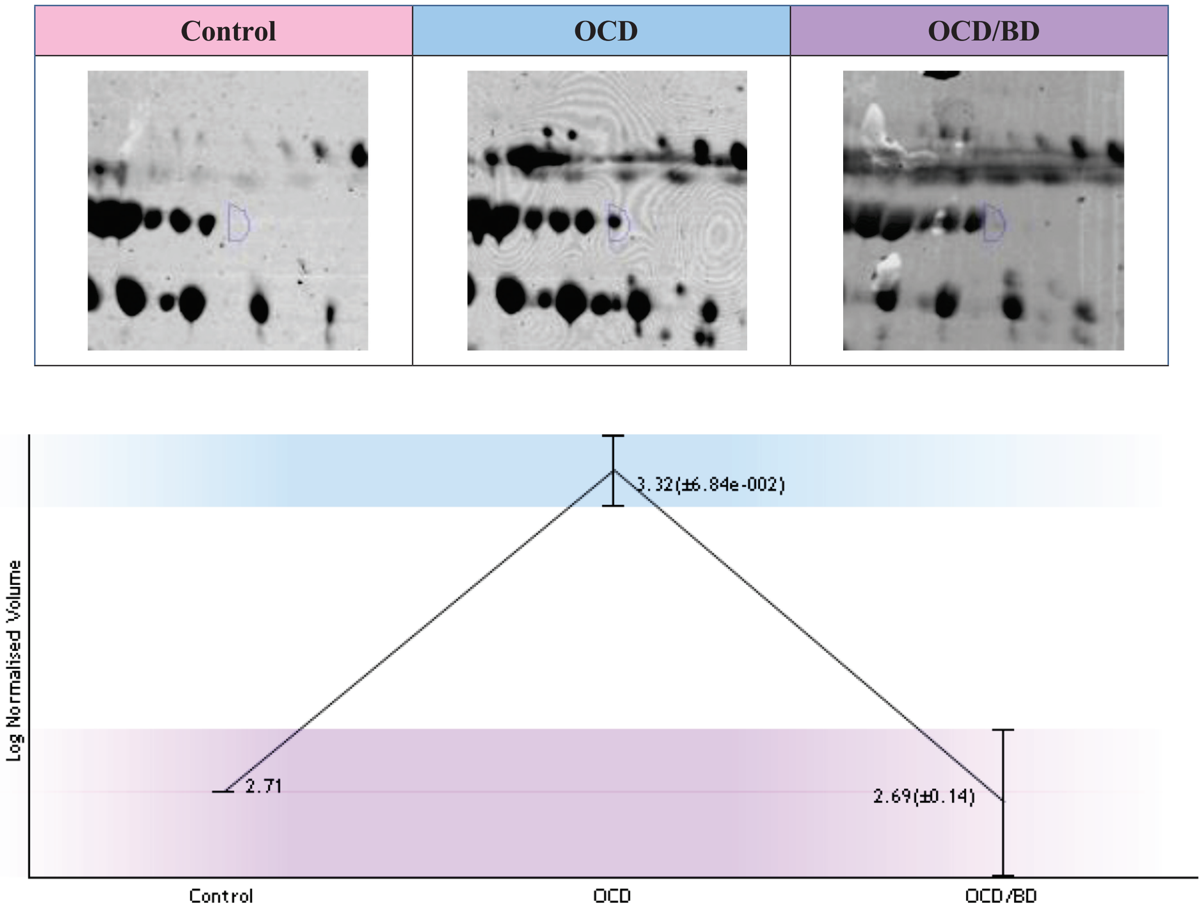


***Figure1*. *The** normalized expression changes of GC in the three conditions of healthy, OCD, OCD/bipolar samples.*

Position (426, 373)
Notes

🟩 Fold>=1.5

🟥 p<=0.05

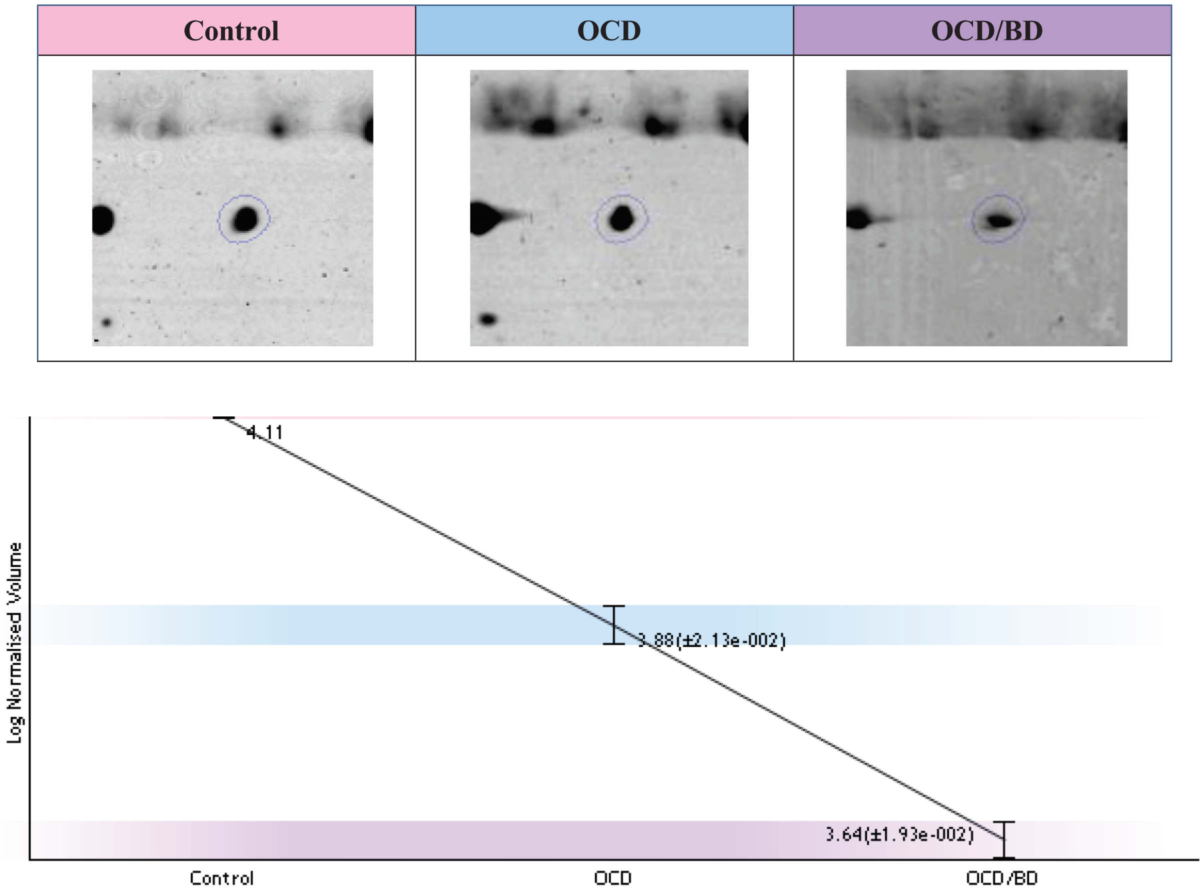


Figure2*. *The* *normalized expression changes of HP in three conditions of conditions of healthy, OCD, OCD/bipolar samples.

Mass spectrometry information related to the two proteins identifications are shown in table 2.

Table2. **The** mass spectrometry analysis and identification with MASCOT of two designated proteins in OCD/Bipolar samples.

Protein Name	Uniprot Code	Protein Seq Coverage	Peptide Matches	Matching Score
Vitamin D binding Protein	P02774	12%	7	434
Haptoglobin	P00738	11%	6	561

Interaction annotation of the identified differentially expressed proteins in OCD samples compared with OCD/bipolar samples. (See figure 3)

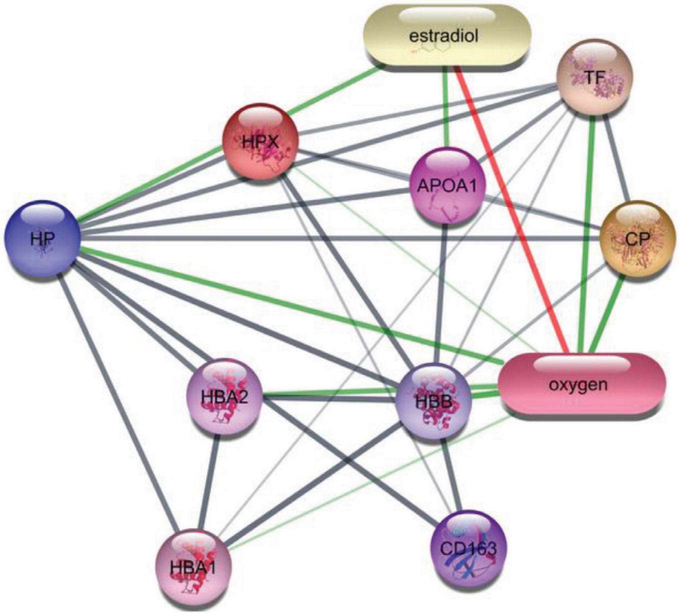
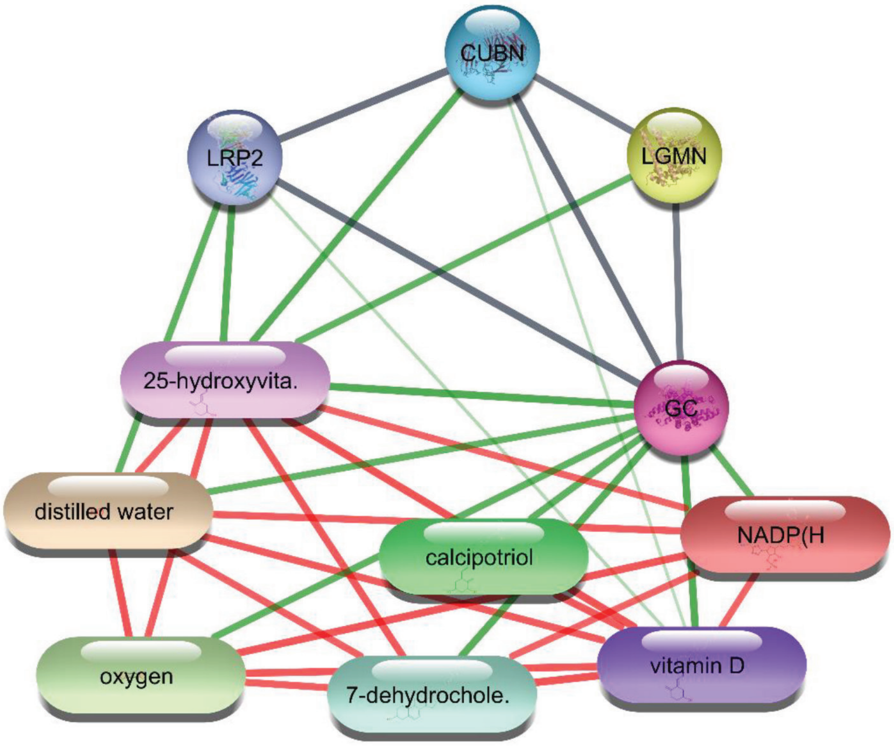


Figure 3. *Interaction analysis of HP protein in a whole interacting pattern indicate presence of eight proteins and two compounds around it when considering number of surrounding nodes of 10. Haptoglobin is the node with the blue color. Links in green, red, and grey are the connections between proteins-compounds, compounds-compounds, and proteins-proteins, respectively. Number of nodes: 11, Number of edges: 34*



Figrue 4. *Interaction analysis of GC protein in a whole interacting pattern indicate presence of one protein and 7 compounds around it when considering number of surrounding nodes of 10. Links in green, red, and grey are the connections between proteins-compounds, compounds-compounds, and proteins-proteins, respectively. Number of nodes: 11, Number of edges: 34*

Discussion

No accurate diagnostic test and treatment approach exited for OCD and its comorbidities (12). On the other hand, the molecular basis of OCD/BP is still unknown. While there are extensive genetic studies for OCD, the consistency is unacceptable across these studies (13). It is known that there is a complex interaction between genes and environmental factors. Proteomics, the relevantly developing field of mental disorder investigation, has shown promising results for identifying the molecular changes at the proteome levels. As mentioned earlier, one of the important sources of human proteome evaluation is serum. This study compared the proteome profile of women with OCD, OCD/BP, and healthy individuals. Among the differentially expressed proteins, two proteins are studied in this paper: GC and HP. These proteins show different behavior and trend changes in expression in the OCD/BP and OCD samples compared to healthy controls. As indicated in Table 1, the expression values of each protein in the three different conditions can be realized. The amount of expression alterations can be understood via fold change, and their significance is considered $p<0.05$ in this study. The pattern of changes in GC and HP are indicated in Figures 1 and 2, respectively.

As it can be inferred, while GC levels show an increment in OCD samples, it decreases in OCD/BP types. This trend change in the comorbidity indicates that the expression pattern of GC differs from OCD to OCD/BP. That is, it is not differentially changed in expression in OCD/BP samples compared to healthy controls. It is a one-fold change difference and below the assigned cutoff. Thus, GC could be a potential biomarker to differentiate OCD and OCD/BP.

Moreover, vitamin D deficiency has been reported to contribute to OCD development risk (14), possibly due to GC serum expression changes. On the other hand, following HP in our three conditions, a similar trend in expression is present. HP demonstrates similar expression changes in OCD/BP compared to OCD samples. In which the expression changes are higher in reduction. The linkage between HP and OCD is also confirmed by our previous study (15).

Furthermore, HP's role is well-documented in mental disorders such as panic disorder and schizophrenia (16, 17); however, it has not been reported for OCD comorbid with BP. This finding supports the potential role of HP in this category of disorders. Further examination of these two spots in an interactome pattern could provide more information about their vital task and the impact of expression changes on other elements in the interactome. The analysis in Figure 3 for HP shows that this protein interacts closely with other vital proteins. Moreover, the presence of oxygen and estradiol (as in Figure 3) suggests that expression alteration of HP possibly influences the level of these compounds, which requires further investigation. It has been reported that estradiol improves OCD outcomes by influencing the serotonin system (18). As mentioned, the relationship between HP and estradiol indicates that the dysregulation of this protein may cause estradiol level impairment, which correlates to OCD symptoms. Moreover, GC, the other altered protein in the serum of OCD patients, shows a correlation with compounds and proteins that could be important in OCD pathology. One crucial compound is vitamin D; this vitamin's low level has been reported in OCD and other psychiatric disorders (19-21).

Overall, the protein expression pattern of the two studied protein spots differed in OCD and OCD/

BP. In a way, the expression of HP is continued with similar patterns and different values from OCD to OCD/Bipolar sample.

In Conclusion

The HP expression pattern deteriorates as the disease progresses. Moreover, a similar manifestation is evident in OCD subjects. This paper underlies one of the differential molecular properties of OCD versus OCD/BP, in which HP and GC may be applied as differential biomarkers of OCD compared to OCD/BP comorbidity, one in the expression level and another in the expression pattern. After extensive validation studies, these protein spots and possibly overall protein levels could be used for future diagnostic approaches.

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The designated patients gave written informed consent before the sampling process. The ethical code for this experiment is IR.SBMU.REC.1393.299.

Author's contribution

Noorollah Tahery conceived the study and participated in the study design, data collection, and data analysis. Mostafa Rezaei Tavirani and Mona Zamanian Azodi wrote the manuscript. Mostafa Hamdieh, Mohammad Rostaminejad, Nahid Mahmooodi participated in data collection and data analysis. All the authors have read and approved the final submitted manuscript.

Conflicts of interest

None

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