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Chemometric based classification study on omega-3 fatty acids in psychotic disorders: schizophrenia , major depressive disorder and suicide attempted

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Abstract

Background and aim: It is supposed that omega-3 fatty acids impacts on psychiatric disorders, in this study we evaluated the impact of erythrocyte omega-3 fatty acids compositions on psychotic disorders: schizophrenia, major depressive disorder and suicide attempted using chemometric based classification.

Materials and Methods: In 125 patients with schizophrenia, major depressive disorder, suicide attempted and healthy control, we used Varian gas chromatography instrument, utilizing an omega wax 250 capillary column (30m× 0.25mm I.D.) and flame ionization detector to measure erythrocyte membrane n-3PUFAs composition and analyzed based chemometric classification.

Results: 125 patients (51 male, 74 female) more than 15 were evaluated. In the control group the level of n-3 PUFA and subtypes of n-3 PUFA significantly was more than other groups. Correlations between subtypes 22:6n-3(DHA), 20:5n-3(EPA), 22:5n-3 and Beck Depression Inventory scale (BDIS) was significant. This study compares results obtained with chemometric methods: SIMCA and PLS-DA. The PLS-DA prediction was better than SIMCA in MDD and suicide attempted. In both techniques, the prediction rate for schizophrenia was 100%.

Conclusion: We revealed the level of erythrocyte omega-3 fatty acids compositions in patients with schizophrenia, major depressive disorder and suicide attempt was lower than healthy population, moreover regarding chemometric methods, PLS-DA was slightly better than SIMCA.

Keywords: erythrocyte, omega-3 fatty acids compositions, psychiatric disorder, schizophrenia, major depressive disorder, suicide, chemometric

Introduction

Several studies have indicated a significant association between abnormal metabolism of phospholipids and fatty acids with the most important psychiatric disorders such as schizophrenia, depression [1, 2] and suicide attempt [3-5]. Furthermore, some experimental studies revealed the therapeutic impact of omega-3 on the treatment of psychiatric illness [6]. The fatty acid intake impacts on neuronal cell membrane phospholipids and modify the mechanisms of electrical signal transduction via modifying ion channel functions. Two important derivatives of omega 3 fatty acid in neuronal phospholipids are Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) [7]. (EPA) and (DHA) regulate the components of neuronal membrane and impacts on neurotransmission, for instance, the higher level of omega-3 PUFAs results in the higher fluidity of the membrane that increases the level of serotonin transport in the membrane [8]. Moreover (EPA) and (DHA) block the signal of protein kinase C and the calcium influx into the cell [8, 9].

Additionally, omega- 3 fatty acids influence on dopamine and serotonin and consequently they may useful in the treatment of patients with schizophrenia, depression [10, 11] and anxiety [12]. Erythrocyte membrane EPA+DHA composition indicates the amount of fatty acid intake in last 2months [13-15].

Chemometric methods are techniques to manage and interpret chemically-derived data and are used in analytical chemistry and metabolomics [16]. In this study we evaluated the impact of erythrocyte omega-3 fatty acids compositions on psychotic disorders: schizophrenia, major depressive disorder and suicide attempted using chemometric based classification to advance the state of the art in analytical instrumentation and methodology in the treatment of psychiatric illness.

Materials and methods

Study subjects and sample preparation for PUFA analysis 125 patients including 29 major depressive disorders (MDD), 30 suicides attempted, 29 schizophrenia patients and 37 age-matched healthy controls were enrolled in this case-control study. The psychiatric disorders were diagnosed based on DSM-IV criteria, moreover, in suicide attempt patients the severity of depression was evaluated according to Beck depression inventory scale (BDIS). Erythrocyte membrane n-3PUFAs composition was measured by Varian gas chromatography instrument, utilizing an omega wax 250 capillary column (30 m × 0.25 mm I.D.) and flame ionization detector (Supplementary data).

Materials and reagents

Samples and standards were systematically protected from contamination by natural existing fatty acids or detergents and from auto-oxidation conducted by direct light and oxygen in air as well during sample processing. The main used reagents were boron trifluoride–methanol complex for synthesis, ultra-pure methanol, chloroform, and n-hexane for extraction step were purchased from Merck (Darmstadt, Germany). Butylated hydroxyl toluene (BHT) and C23:0 fatty acid were added to the samples (1µg per 1ml of sample) as an antioxidant reagent and internal standard respectively. Methyl ester standard mixtures, with known identity and quantity of PUFA components (EPA, 18:3n-3, 22:5n-3 and DHA) (C4-C24) were provided from Bellefonte, USA. Single component standards are also used for qualitative analysis and quantitative determination in real samples.

Gas chromatography

The blood samples were taken from all patients and transferred to an EDTA-containing vacationer tube kept in an Ice bath. After centrifugation, the plasma and buffy coat were removed. The erythrocytes were washed 3 times in 0.9 %w NaCl and kept at -70 °C till the analysis phase. Lipid extraction was performed by cells homogenization in Chloroform /methanol (1:2 vol. /vol.) containing 5 mg/100 ml butylated hydroxyl Toluene (Darmstadt, Germany) as an antioxidant and 5 mg/100 ml fatty acid (22:0) as an internal standard. In the next step, the Gas chromatography technique was performed for quantitative analysis of PUFAs according to the procedure described previously by Kang and Wang. 21 In brief, fatty acids are converted into methyl esters by heating with BF3 in Methanol, which is separated and concentrated under nitrogen. Fatty acid methyl Esters were analyzed by Varian gas chromatography instrument, utilizing an omega wax 250 capillary column (30 m × 0.25 mm I.D.) and flame ionization detector. Quantitative analysis was performed with the aid of chromatin GC solution software package. Individual fatty acid methyl esters were identified by comparing retention times with authentic standards. Values were expressed as a percentage of weight (%W). PUFA values were represented as the mean levels of each fatty acid in mg/100mg of total lipid Species. In this study, we compared results obtained with two chemometric methods SIMCA and PLS-DA.

Statistical Analyses

Data were analyzed using SPSS version 20. Categorical data are presented as numbers (%), and continuous data as mean ± SD. We used the Chai_2 or Fisher's exact test to compare categorical variables and the Student's t test to compare continuous variables $\alpha < 0.05$ was considered significant

Results

125 patients including 51 male and 74 female more than 15 were evaluated. In the group with suicide attempt 9 patients (31%) used chemical materials and 18patients (62%) used mechanical methods, moreover the suicide attempt in 16 patients (55.17%) was impulsive and in 19 patients was planned. In the control group the level of n-3 PUFA significantly was more than other groups. Moreover, the subtypes of n-3 PUFA in the control group were significantly more than MDD, schizophrenia and suicide attempt groups (table 1). Correlations between subtypes 22:6n-3(DHA), 20:5n-3(EPA), 22:5n-3 and Beck Depression Inventory scale (BDIS) was significant (Table 2). This study compares results obtained with chemometric methods: SIMCA and PLS-DA. The PLS-DA prediction was better than SIMCA in MDD and suicide attempted. In both techniques, the prediction rate for schizophrenia was 100% (table 3), (figures 1-7).

Table 1: Erythrocyte n-3 polyunsaturated fatty acids (PUFA) composition in healthy subjects and three groups of patients

n-3PUFA	Healthy controls Mean ± SD	Schizophrenia Mean ± SD	MDD mean ±SD	Suicide attempted Mean ± SD	*P value*
18:3n-3	0.18±0.02	0.11±0.02	0.16±0.02	0.10±0.02	0.031
20:5n-3(EPA)	1.1±o.46	0.43±0.02	0.99±0.43	0.53±0.18	0.0003
22:5n-3	2.14±0.47	1.17±0.34	1.96±0.57	1.14±0.34	0.0001
22:6n-3(DHA)	4.77±1.03	2.49±0.68	2.57±0.69	2.48±0.68	0.001
22:6n-3/22:5n-3	2.23±0.83	2.11±0.59	2.24±1.25	2.17±0.59	0.032
n-3 PUFA	8.01±1. 45	4.20±0.96	5.68±1.02	4.25±1.01	0.001

Table 2: Correlations between RBC fatty acid levels and Beck Depression Inventory scale (BDIS)

Fatty acid	MDD (n = 29)		Suicide(n = 30)	
	r	P values	*r*	*P values*
22:6n-3(DHA)	- 0.74	0.001	- 0.74	0.001
18:3n-3	- 0.47	0.421	- 0.36	0.532
20:5n-3(EPA)	- 0.72	0.001	- 0.72	0.001
22:5n-3	- 0.68	0.005	- 0.69	0.005

Table 3: The results of model validation for SIMCA (Soft Independent Modeling of Class Analogy) and PLS-DA (Partial least squares - Discriminant Analysis) methods and the prediction of testing set samples using both methods

Classes	Model Validation		Prediction	
	PLS-DA	SIMCA	PLS-DA	SIMCA
Healthy subjects	92.6%	88.89%	90%	90%
MDD*	68.42%	63.16%	90%	70%
Suicide attempted	75%	80%	80%	70%
Schizophrenia	73.7%	84.2%	100%	100%

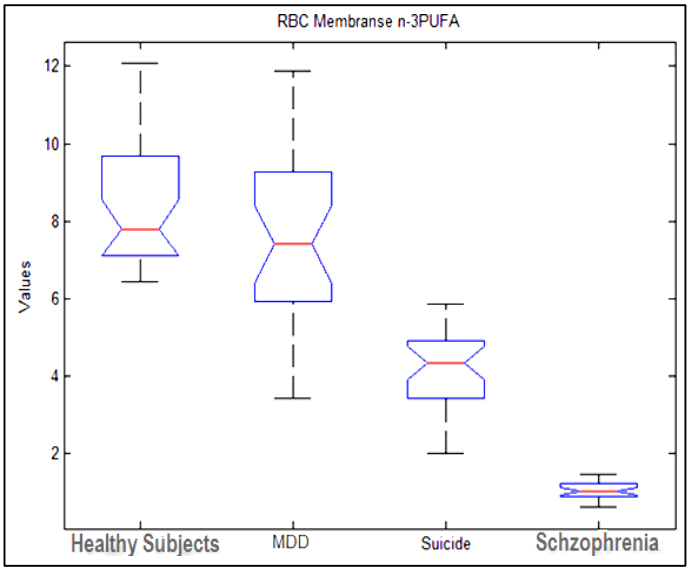

Fig 1: ANOVA Box plot of four classes of chromatograms

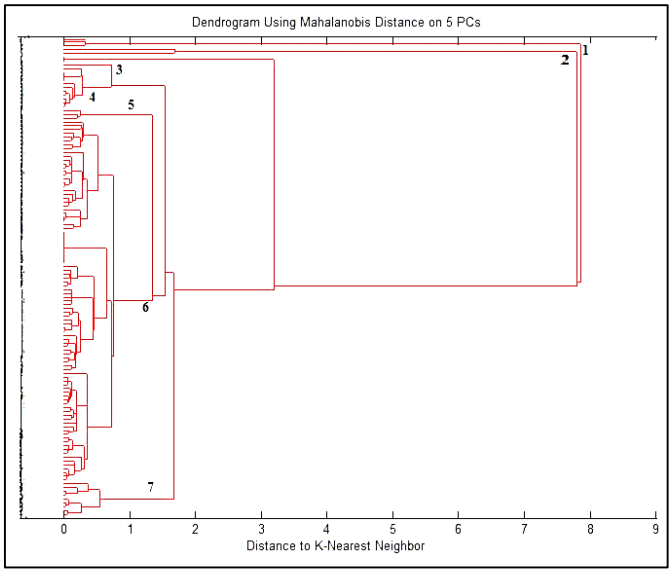

Fig 2: The dendrogram of cluster analysis

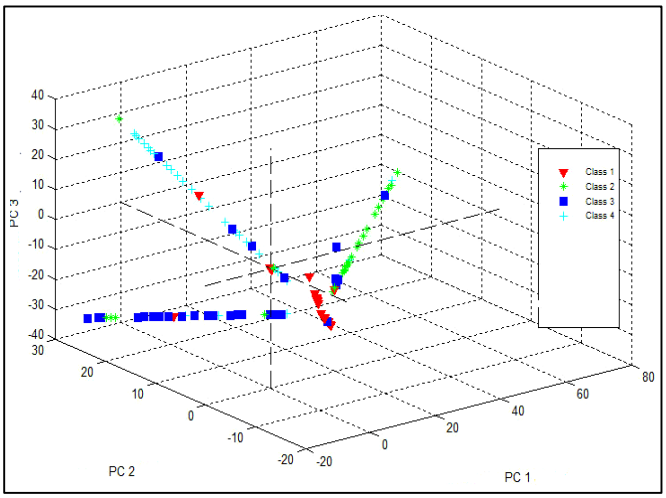

Fig 3: Three first principle components obtained by principal component analysis. Healthy subjects (class 1), Major depression disorder (class 2), suicide attempt (class 3) and schizophrenia (class 4).

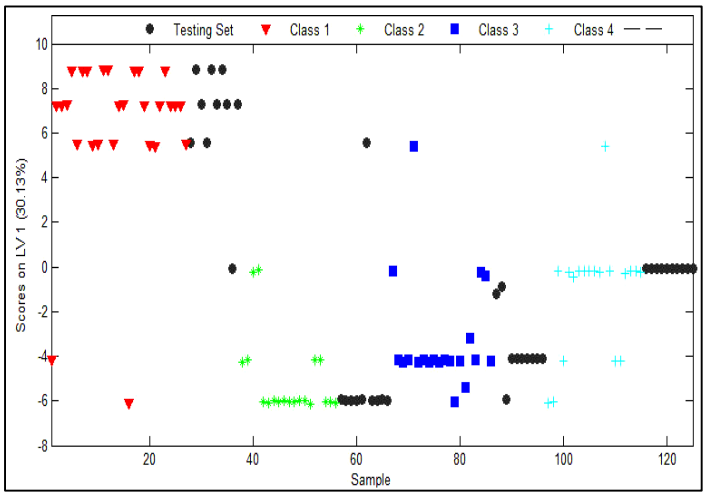

Fig 4: Training and testing set for Healthy controls, MDD, Suicide and Schizophrenia patients by PLS –DA model

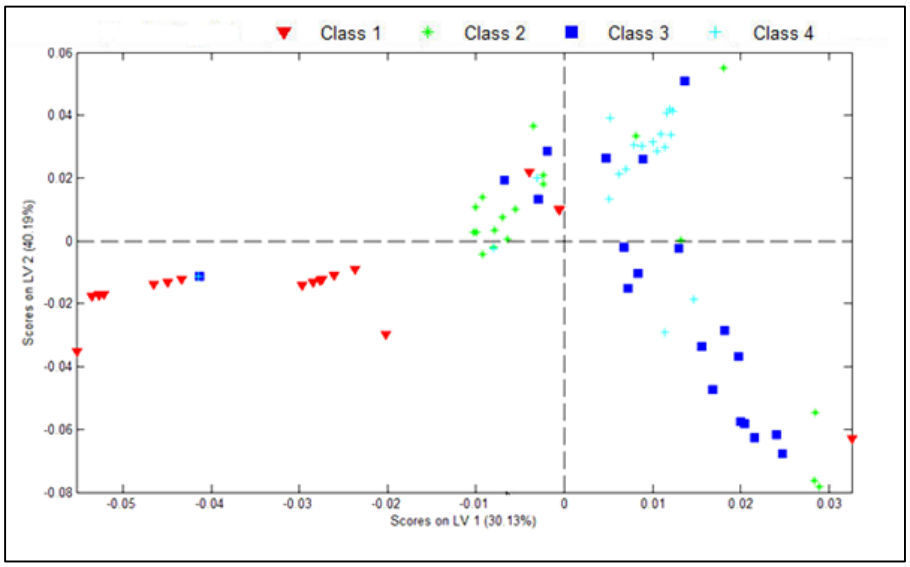

Fig 5: PLS – DA Score plot for four Classes; healthy subjects (class 1), Major depression disorder (class 2), suicide attempt (class 3) and schizophrenia (class 4).

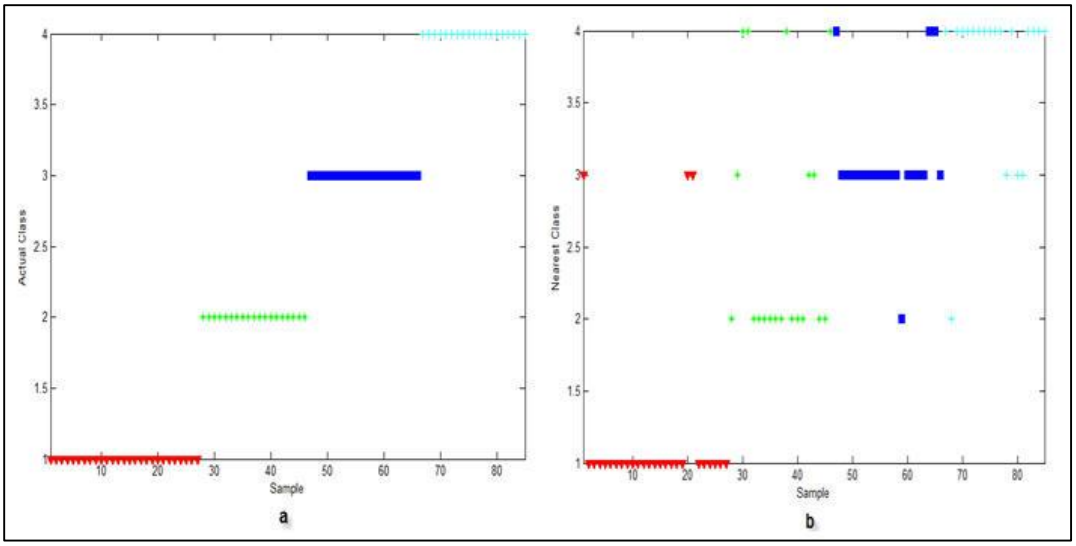

Fig 6: Actual class (a) and prediction of training set by PCA model (b)

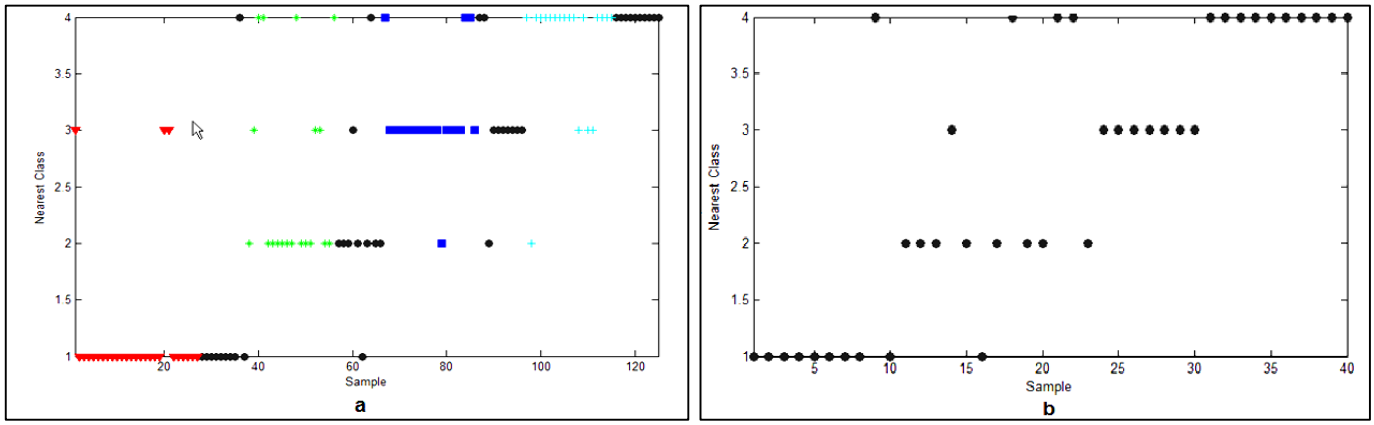

Fig 7: Training and testing set samples (a) and testing set sample (b) predicted by SIMCA model

Discussion

It is well established that omega-3 fatty acids are involved in several functions such as nerve membrane neuroplasticity and synaptic transmission [17], and some trials revealed fatty acids improve mood disorders [18, 19] ADHD [20] and manic symptoms [21] in children increase the level of RBC EPA+DHA [22]. In this regard Zarrini *et al*. in 2003 revealed 1g of EPA improved the symptoms of aggression and depressive in patients with borderline personality disorder [23]. Also, Merck *et al*. in 2004 investigated that EPA as an adjunct agent significantly impacts on refractory depression [24]. We evaluated 125 patients more than 15 years, in the control group the level of n-3 PUFA significantly was more than other groups. Moreover, the subtypes of n-3 PUFA in the control group were significantly more than MDD, schizophrenia and suicide attempt groups. Correlations between subtypes 22:6n-3(DHA), 20:5n-3(EPA), 22:5n-3 and Beck Depression Inventory scale (BDIS) was significant. This study compares results obtained with chemometric methods: SIMCA and PLS-DA. The PLS-DA prediction was better than SIMCA in MDD and suicide attempted. In both techniques, the prediction rate for schizophrenia was 100%. Hedelin *et al*. supported these findings and revealed that low fatty acids intake relates to more risk of psychotic-like symptoms (26). Moreover Amminger *et al.* revealed that n-3 fatty acids treatment may prevent some psychiatric disorders [27]. In agreement with current practice Gracious *et al*. investigated that treatment of patients with bipolar disorder with n-3 fatty acids improve the symptoms of disease [28]. Additionally, erythrocyte membrane fatty acid ω-6 to ω-3 ratio associated with improvement of psychiatric disorder [29]. These findings specified that fatty acids impact on the pathophysiology of psychiatric disorders, however, the results of studies are not consistent and some studies have provided conflicting results. For instance Hamazaki *et al.* in 2015 did not show any relationship between PUFAs or other fatty acids and schizophrenia, bipolar disorder, major depressive disorder and suicide attempt [30]. Another study also did not recommend omega-3 PUFA for the treatment of patients with psychiatric disorders and emphasized that more studies are required to validate the efficacy of fatty acids [31]. To the best of our knowledge, this was the first study using chemometric based classification to evaluate fatty acids on psychiatric illness. We showed that two methods PLS-DA and SIMCA were the same regarding the prediction of schizophrenia, although in MDD and suicide attempted PLS-DA was slightly better than SIMCA.

Small sample size was the most important limitation of current experience further studies with larger series using chemometric methods are suggested.

Conclusion

We revealed the level of erythrocyte omega-3 fatty acids compositions in patients with schizophrenia, major depressive disorder and suicide attempt was lower than healthy population, moreover regarding chemometric methods, PLS-DA was slightly better than SIMCA.

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