Altered Levels of Serum Haptoglobin and Apo A-I in Schizophrenia
(Perubahan Paras Haptoglobin dan Apo A-I Serum dalam Skizofrenia)

Tze Jen Chow & Han Chern Loh*

ABSTRACT

Schizophrenia is a debilitating psychiatric syndrome that affects 1% of the world's population. Abnormalities in immune functions and role of inflammatory markers have been widely described in schizophrenia. With the alarming high prevalence, this study aims to investigate the association of acute phase proteins with schizophrenia. We investigated the serum proteome of 20 schizophrenic patients and 20 healthy controls using two-dimensional gel electrophoresis and mass spectrometry. The spots were analysed using Image Master 2D Platinum software. In total, we have detected 774 protein spots in human serum and found that three of them showed altered changes in schizophrenic patients, as compared to healthy controls. Among these acute phase proteins, haptoglobin (p = 0.003) and two isoforms of apolipoprotein A-I (p = 0.004, p = 0.003) were found to be significantly decreased in patients, as compared to controls. Our findings support the hypothesis that inflammatory response system is linked to the pathophysiology of schizophrenia.

Keywords: Apolipoprotein A-I; haptoglobin; schizophrenia

ABSTRAK

Skizofrenia adalah sejenis penyakit psikiatrik yang melesukan dan mempengaruhi 1% daripada penduduk sedunia. Ketidaknormalan dalam fungsi pertahanan dan peranan sebagai penanda keradangan semakin meluas dihuraikan dalam skizofrenia. Dengan bahaya yang berpeluang tinggi dijangkiti, kajian ini bertujuan untuk menyelidik hubungan protein fasa tajam dengan skizofrenia. Kami mengkaji proteom serum 20 pesakit skizofrenia dan 20 kawalan yang sihat dengan menggunakan gel elektroforesis dua-dimensi dan spektrometri jisim. Tanda-tanda dianalisiskan dengan menggunakan perisian ImageMaster 2D Platinum. Secara kesuluruhannya, kami telah mengesan 774 tanda protein dalam serum manusia dan mendapati tiga tanda menunjukkan perubahan dalam pesakit skizofrenia berbandingkan dengan kawalan yang sihat. Di kalangan tiga protein fasa tajam ini, haptoglobin (p = 0.003) dan dua isofom apolipoprotein A-I (p = 0.004, p = 0.003) didapati menurun secara nyata dalam pesakit berbanding dengan kawalan. Keputusan ini menyokong andaian bahawa sistem tindak balas keradangan adalah berhubung kepada patofisiologi skizofrenia.

Kata kunci: Apolipoprotein A-I; haptoglobin; skizofrenia

INTRODUCTION

Schizophrenia (SCZ) is a debilitating mental disorder that affects approximately 1% of the world's population (Schwarz & Bahn 2008). From 2003 to 2005, there were 7151 cases that had been registered in Malaysia (National Mental Health Registry 2006) and 1.8% of the total suicide death in year 2007 was caused by SCZ (Hayati et al. 2008). In spite of the high prevalence and morbidity, its etiology remains unknown and usually associated with various factors including genetic inheritance, oxidative stress and immune alterations (Morera et al. 2007; Othmen et al. 2008).

Acute phase proteins (APPs) are markers of inflammation, whose plasma level alters with the activation of inflammatory response system (Gaur et al. 2008). The importance of APPs as biomarker have been reported in diseases such as cancer (Chen et al. 2009), depression and Alzheimer's disease (AD) (Harr et al. 1996). During abnormal immune reactions, inflammatory cytokines stimulates the production of APPs such as haptoglobin, apolipoproteins and macroglobulin to activate the complement system (Greco et al. 2009). In the central nervous system, this inflammatory process might impair the blood-brain barrier and cause damage to the brain (Yang et al. 2006).

Abnormalities in the immune function and the role of inflammatory markers have been widely described in SCZ (Maes et al. 1997; Morera et al. 2007). Alterations in the plasma APP levels of schizophrenic patients suggested possible role of the proteins in the pathophysiology of SCZ (Wan et al. 2007). Previous researches on APPs in SCZ (Table 1) mainly focused on haptoglobin (Hp), apolipoprotein (Apo) A-I, Apo A-IV and Apo D (Rothermundt et al. 2001).

Hp is a free haemoglobin-binding protein where its low level is associated with hemolysis, allergy and hepatocellular disorders (Sadrzadeh & Bozorgmehr 2004). In SCZ, Hp plays a role in the oxidative injury of the central nervous system (CNS) (Wan et al. 2007), in which increased oxidative stress was hypothesised in the etiology of SCZ (Kunz et al. 2008; Othmen et al. 2008).

TABLE 1. Biomarkers for SCZ identified by proteomics methods using human samples.

Protein marker	Sample type	Observations	References
Apo A-I	brain tissue	decreased	Huang et al. (2008)
	CSF	decreased	Huang et al. (2008)
	red blood cells	decreased	Huang et al. (2008)
	plasma	decreased	Huang et al. (2008); La et al. (2007); Yang et al. (2006)
Apo A-IV	CSF	decreased	Jiang et al. (2003)
	plasma	increased	Yang et al. (2006)
Apo D	plasma	increased	Mahadik et al. (2002)
	plasma	decreased	Thomas et al. (2001)
	brain tissue	increased	Thomas et al. (2001)
Apo E	brain tissue	increased	Dean et al. (2003)
Haptoglobin	plasma	increased	Maes et al. (1997); Wan et al. (2007); Wong et al. (1996); Seal & Eist (1966); Yang et al. (2006)
α1-antitrypsin	plasma	increased	Wong et al. (1996); Yang et al. (2006)
Complement factor B	plasma	increased	Yang et al. (2006)
Transthyretin	CSF	no relationship	Huang et al. (2007)
	plasma	decreased	Yang et al. (2006)

Apolipoproteins have been widely studied in SCZ. Apo A-I is synthesised predominantly in the liver and intestine, involving in the transport of cholesterol from tissues to the liver (Rottman et al. 1991). In humans, the gene of Apo A-I is found to overlap with SCZ susceptibility loci on chromosome 11q23 (Arinami et al. 1990), and there is accumulating evidence indicating that down-regulation Apo A-I protein is often linked with the pathology of SCZ (Huang et al. 2008; La et al. 2007). Based on the immune-inflammation hypothesis, it has been shown that schizophrenic patients treated with the combination of antipsychotics and lipophilic anti-inflammatories would lead to down-regulation of immune response in the CNS (Muller et al. 2002). Thus, the aim of this study was to investigate the association of APPs with SCZ.

MATERIALS AND METHODS

SUBJECT SELECTION

The sample population consisted of 20 controls with no history of psychiatric disorders (10 male and 10 female) and 20 schizophrenic subjects (10 male and 10 female) recruited from Bahagia Ulu Kinta Hospital, Perak, Malaysia. This study was approved by the Medical Research and Ethics Committee, Ministry of Health, Malaysia. Written consents were obtained from healthy subjects before participation in the study. The diagnoses of schizophrenic subjects were done according to the Mini International Neuropsychiatric Interview (M.I.N.I.), English Version 5.0.0 (Sheehan et al. 1998), by treating psychiatrists. Only patients of paranoid subtype were selected. Both patients and controls were residents of Malaysia, age ranging from 30 to 45 years (patient mean age = 38.55±5.33; control mean age = 37.00±4.68).

In total, 10 mL of peripheral blood were ejected from subjects into 10 mL vacutainer (Becton, Dickinson & Company, USA) for serum collection. The blood samples were span down at 4000 rpm for 5 min at 4°C, and serum at the supernatant layer was collected and stored at -80°C. Total protein content was determined by Bradford assay.

TWO-DIMENSIONAL GEL ELECTROPHORESIS (2-DE)

First dimension separation by IEF was performed using Ettan IPGphor 3 system (GE Healthcare, USA), followed by SDS-PAGE using Hoefer SE 600 Ruby (GE Healthcare, USA) for second dimension separation. In total, 50 μg of serum protein was rehydrated overnight at room temperature with rehydration buffer (7 M urea, 2 M thiourea, 4% CHAPS, 20 mM DTT, and 0.5% IPG buffer) to a final volume of 250 μL on 13 cm, pH 4 – 7 Immobiline DryStrip gels (GE Healthcare, USA). IEF was performed at 500V for 1 h, ramped up to 1000V for 1 h and then maintained at 8000V for 2 h. The strip was equilibrated (6 M Urea, 50 mM Tris–HCl pH 8.8, 30% glycerol, 2% SDS) with 10 mg/mL DTT, followed by 25 mg/mL iodoacetamide. Second dimension was performed on 12.5% SDS-polyacrylamide gel (18 × 16 cm², 1 mm thickness), and stained using PlusOne Silver Staining Kit (GE Healthcare, USA).

SPOT ANALYSIS

ImageMaster 2-D Platinum software (GE Healthcare, USA) was used for the analysis of the gels. After spot detection and gel matching, differently expressed spots were identified. For peptide mass fingerprinting (PMF), a preparative gel was done and stained using Coomassie blue R-250, where spots were excised and sent for trypsin digestion and MALDI-ToF/ToF MS. The database search was done using Mascot 1.9 (www.matrixscience.com)

where Mascot scores greater than 82 are significant (p < 0.05) for PMF search. Data were presented as mean ± standard deviation. Differences between control and patient were assessed using one-way analysis of variance where p-value < 0.01 was considered statistically significant.

RESULTS

Human serum proteins were quantified and identified from 2-DE gels using the ImageMaster 2-D Platinum software and MALDI-ToF/ToF MS. A total of 774 protein spots were detected by the software on silver-stained gels. To fulfil the acceptable criteria for disease biomarkers in differential display techniques, only protein spots with expression levels of two-fold variances were selected (Yang et al. 2006). A comparison of the 2-DE gels of patients' serum with those of healthy controls indicated that some spots were significantly varied. Three clear protein spots were identified using the PMF method of MALDI-ToF/ToF MS (Figure 1). Table 2 lists the Swiss-Prot accession numbers and full names of the protein spots, as well as their mascot score, molecular mass, pI values, expectation level, and protein amino acid sequence coverage by matching peptides. The expression levels and fold-change of the proteins are listed in Table 3. The expression of Hp (spot 1) in controls increased dramatically by 5.64-fold compared to that of patients. Similarly, Apo A-I-1 (spot 2) and Apo A-I-2 (spot 3) show 2.24-fold and 4.21-fold higher expression respectively in controls. Hp (p = 0.00307) and Apo A-I (p = 0.00365) displayed a significant decreased in expression levels in the serum of the patients compared to controls. Other spots did not show significant quantitative alteration between control and patient.
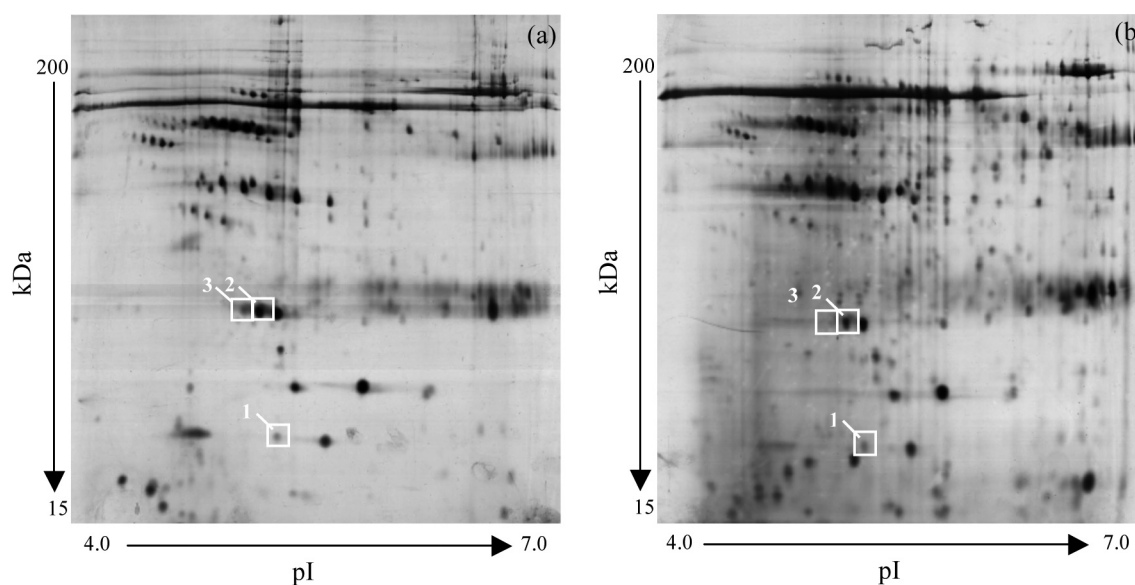

FIGURE 1. Two-DE map of (a) control serum proteins and (b) patient serum proteins. The gel was separated on 18×16 cm^2 plate and silver-stained. The horizontal axis represents the IEF dimension, which stretches from pH 4 to 7. The vertical axis represents 12.5% SDS-PAGE gel. Arrows indicate differentially expressed proteins identified by MS

TABLE 2. Mass spectroscopy identification of differentially expressed spots

Spot	Protein name	Swiss-Prot no.	Mascot score	MW (kDa)	pI	Coverage (%)	Expect
1	Haptoglobin	P00738	391	38.72	6.14	10.0	7.3e-033
2	Apolipoprotein A-I-1	P02647	391	28.06	5.17	71.0	7.3e-033
3	Apolipoprotein A-I-2	P02647	197	28.06	5.27	65.0	1.8e-013

TABLE 3. List of the differentially expressed proteins

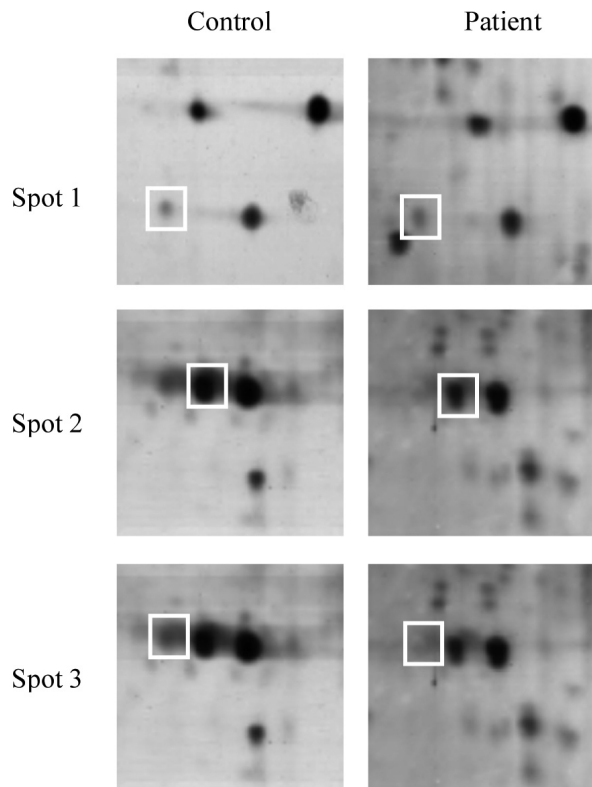| Protein name	Protein expression (% volume)			
	Control	Patient	p-value	Fold change
Haptoglobin	0.91 ± 0.89	0.16 ± 0.13	0.003	5.64
Apolipoprotein A-I-1	2.51 ± 1.60	1.12 ± 0.55	0.004	2.24
Apolipoprotein A-I-2	1.00 ± 0.85	0.24 ± 0.29	0.003	4.21

Control Patient

FIGURE 2. Enlarged panels of significant difference protein patterns in controls and patients

DISCUSSION

This study demonstrated the use of proteomic analysis of human serum in investigating the pathology of SCZ. In this study, we found the down-regulation of both serum Hp and Apo A-I levels in patients as compared to healthy controls.

Most investigations that studied the relationship between Hp and SCZ have found elevated levels of Hp in schizophrenic patients (Wan et al. 2007; Wong et al. 1996). However, our results showed significant down-regulation of Hp in schizophrenic patients. Difference in sample population may contribute to the different results (Wan et al. 2007), in which our study only selected patients of paranoid subtype. Genetic study of the Hp gene suggested that various SCZ subtypes are associated with different Hp phenotype frequencies. Patients with disorganised, undifferentiated and residual subtypes show an excess of the Hp 2-2 phenotype but paranoid patients did not fall into the pattern (Maes et al. 2001). Although Hp phenotyping is not performed in our study, this finding might be related the decreased patient Hp level found in the paranoid patients.

SCZ patients were shown to have lower carotenoid level compared to controls (Chow et al. 2010), where carotenoid serves as indicator of overall antioxidant level in human (Svilaas et al. 2004; Zhao et al. 2003). Hp has an additional role as antioxidant, thus the reduced Hp level in the patient serum may be associated with the low antioxidant level seen in SCZ (Sadrzadeh & Bozorgmehr 2004).

Apo A-I serves to decrease cellular reactive oxygen species (Robbesyn et al. 2005) and is involved in lipoprotein degeneration and regeneration in the CNS (La et al. 2007). Yang et al. (2006) reported significant down-regulation of Apo A-I in SCZ patient. It is noteworthy that our study observed a similar trend of significant decreased serum Apo A-I in SCZ patients, therefore further confirming association of Apo A-I with the pathology of SCZ. Besides that, the gene for Apo A-I overlaps with SCZ susceptibility chromosome 11, and is also closely linked to the gene for Apo A-VI (Karathanasis 1985), where Apo A-VI is widely reported in SCZ (Jiang et al. 2003; Yang et al. 2006).

Apo A-I is shown to be associated with transthyretin (TTR), in which interaction of TTR with high density lipoproteins occur through binding with Apo A-I (Sousa et al. 2000). Due to this positive interaction, it is believed that Apo A-I and TTR will have the same trend in expression level. Decreased levels of TTR are often reported in SCZ (Yang et al. 2006), thus Apo A-I is expected to show a similar trend, as observed in the patient serum of the current study.

As a conclusion, it was shown herein the altered expressions of Hp and Apo A-I in serum of SCZ patients. Our findings suggest decreased levels of Hp and Apo A-I might be linked to the disorder and have the potential to become biomarkers for SCZ diagnosis. This might give a new pointer in the investigation of the pathogenesis of SCZ.

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Faculty of Engineering and Science
Universiti Tunku Abdul Rahman
Jalan Genting Kelang, Setapak
53300 Kuala Lumpur, Malaysia

*Corresponding author; email: hcloh@utar.edu.my