medical sciences forum

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Proceeding Paper

Identification of Potential Protein Biomarkers in a Depressed Chinese Malaysian University Student Using Liquid Chromatography-Tandem Mass Spectrometry †

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† Presented at the 2nd International Electronic Conference on Biomedicines, 1–31 March 2023; Available online: https://ecb2023.sciforum.net.

Citation: Yap, S.Y.; Foo, C.N.; Lim, Y.M.; Ng, F.L.; Tang, P.Y.; Singh, J.K.N.; Sidik, S.M.; Pheh, K.-S. Identification of Potential Protein Biomarkers in a Depressed Chinese Malaysian University Student Using Liquid Chromatography-Tandem Mass Spectrometry. *Med. Sci. Forum* **2023**, *3*, x. https://doi.org/10.3390/xxxxx
Published: 1 March 2023

Abstract: Depression is a serious psychological disorder with high prevalent rates, especially among university students. Serum proteins related to immune system, oxygen and lipid transfer could have contributing roles in the development of depression and could act as biomarkers for depression. Currently, there is a lack of accurate biological methods to diagnose depression. Biomarkers could be an inexpensive and convenient way to predict depression and understand its pathophysiology. This study aimed to screen the serum proteome profile of a depressed student for the identification of potential depression biomarkers. A Malaysian private university student who was recruited from the pre-test study (n = 10) was further analyzed for serum proteome, due to he was depressed with scores of 15 out of 27 on the Patient Health Questionnaire (PHQ-9). After depleting the high-abundance proteins from the serum sample, liquid chromatography-tandem mass spectrometry (LC-MS/MS) was performed to identify the expressed proteins. A total of 224 proteins were identified. Globins, globulins, apolipoproteins and glycoproteins were most commonly detected. Here we show the potential biomarkers to identify depression vulnerable individuals. These findings can be relevant to the development of new diagnostic and treatment strategies. However, further studies with larger sample sizes and healthy controls are needed to confirm the role of these candidate biomarkers for the prediction and diagnosis of depression.

Keywords: depression; depressive disorder; serum proteome; proteomics; biomarker; protein markers; identification; university student; case study; LCMS

1. Introduction

Mental disorder is one of the biggest health issues affecting Malaysians [1]. National Health and Morbidity Survey 2019 reported that nearly half a million of Malaysians suffered from depression [2]. According to a study in 2020, 42.3% of Malaysian adults had depression [3]. University students are more vulnerable to depression compared to other populations. A study in 2018 showed that 29.4% of university students in Malaysia reported to have depression [4]. Pilot study conducted by the authors showed that Malaysian university students had a prevalence of depression of 33.8% [5]. Depression can cause a series of emotional and physical problems that interfere with a student's ability to work, eat, sleep and enjoy life. Resources provided by university to combat mental health problems are limited and has indirectly lead to the increasing prevalence of depression among the students.

For the past few decades, researchers have attempted to link diagnostic and prognostic biomarkers for psychiatric disorders including depression, schizophrenia, and bipolar disorder. Bodily fluids like blood, urine, and cerebral spinal fluid are one of the easily accessible sources to detect these psychiatric biomarkers [6]. For example, serum S100B were found to be lower in medicated youth patients, compared with those who were drug-free, and healthy controls [7]. Besides, decreased serum total proteins, albumin, and globulin were found associated with depressive severity in schizophrenia [8]. In a recent study conducted among Chinese older adults, higher levels of immunoglobulin A, lower levels immunoglobulin M and complement C3 were found in depressed group [9]. The discovery of these biomarkers could provide a better insight of mental illness mechanisms and aid the diagnosis of psychiatric disorders, as well as accelerate the development of targeted therapy tailored to each individual. This study aimed to screen the serum proteome profile of a depressed student for the identification of potential serum protein marker panel for detection of depression.

2. Methods

Written consents were obtained prior to the data collection. Patient health questionnaire-9 (PHQ-9) [10] was used to assess the presence of depression. Blood sample was collected in red vacutainer with no anticoagulants and the separated serum was stored at −80 °C before analysis. The serum was analysed by QT of liquid chromatography with tandem mass spectrometry (LC-MS/MS). Agilent MassHunter data acquisition software and PEAKS 7.0 software were used for protein profiling.

3. Results

Globulins were most commonly detected, followed by globins, apolipoproteins, glycoproteins, inhibitory proteins, complement proteins, binding protein, ceruloplasmin, albumin, and luminal protein.

Type	Protein	−10lgP
Globulin	Alpha-2-macroglobulin	209.22
	Serotransferrin	170.6
	Immunoglobulin	166.94~64.69
	Vitamin D-binding protein	158.89
	Prothrombin	145.5
	Angiotensin	104.31
	Protein AMBP	72.23
	Transthyretin	61.89
Globin	Haptoglobin	127.26
	Hemoglobin subunit beta	87.75
	Hemoglobin alpha	53.68

	Hemoglobin C	53.68
Apolipoprotein	Apolipoprotein B	218.02
	Apolipoprotein H	134.17
	Apolipoprotein A	132.28~71.93
	Apolipoprotein C	70.65~37.24
	Apolipoprotein E	55.66
	Apolipoprotein D	24.17
Glycoprotein	Beta-2-glycoprotein	134.17
	Alpha-2-HS-glycoprotein	132.92
	Hemopexin	124.8
	Vitronectin	117.28
	Antithrombin	102.28
	Alpha-1B-glycoprotein	99.58
	Afamin	88.28
	Alpha-1-acid glycoprotein	87.65~74.17
	Fibronectin	87.14
	Histidine-rich glycoprotein	82.56
	Clusterin	75.45
	Complement factor H	61.76
Complement protein	Complement C3	257.29
	C4a anaphylatoxin	201.95
	C3/C5 convertase	103.31
	Complement factor B	103.31
	Complement component C6	63.39
Binding protein	Hepatitis B virus receptor binding protein	162.98
	Epididymis secretory sperm binding protein	160.87~99.58
	Gelsolin	95.4
	Actin-depolymerizing factor	95.4
	Retinol-binding protein	81.8
	C4b-binding protein alpha chain	81.16
Inhibitory protein	serpins	113.58~65.31
	Inter-alpha-trypsin inhibitor heavy chain H2	125.8
	Inter-alpha-trypsin inhibitor heavy chain H1	120.32
	Inter-alpha-trypsin inhibitor heavy chain H4 (ITIH4) protein	113.23
	Kininogen-1	108.14
	Bradykinin	108.14
Other	Ceruloplasmin	141.67
	Albumin	235.66~225.4
	Luminal protein	169.56~133.23

$^{-10lgP}$ represents the level of significance.

4. Discussion

The functions of these identified proteins are mostly related to our immune system and lipids transport. The contributing biological cause behind the link between immune

system and depression could be due to inflammation. When we are sick, our immune system creates an inflammatory response that makes us feel sad, irritable or unmotivated in order to keep us in bed for our body to devote the body resources to fight and heal. When inflammatory cytokines in body reach a certain threshold, our brain will initiate its own inflammatory response, and make the macrophages pump out cytokines that not only attack invaders like germs, it can harm the healthy tissues throughout body and brain [11]. The cytokines can also alter the neurotransmitter systems involved in the development of depression [12]. Meta-analysis in 2019 demonstrated alterations of cytokines levels in patients with antidepressant outcomes [13].

C-reactive protein (CRP) is an inflammation protein marker and it increases during infection [14]. Numerous studies correlated elevated CRP levels with presence of depression and its severity. Meta-analysis consists of 30 studies with 11,813 participants showed that presence of low-grade inflammation and elevated CRP levels were found in depressed patients [15]. Additionally, higher CRP levels was associated with depressive symptoms from Netherlands Study of Depression and Anxiety (NESDA) and UK Biobank cohorts [16].

Neurotransmitter systems can be affected by inflammation via reduction of monoamine synthesis. Monoamines neurotransmitters like dopamine, norepinephrine, and serotonin are primarily associated with the development of depression [17]. Increased dopamine signalling was found associated with lower levels of depressive symptoms among Asian samples [18]. However, contradictory result was found among Caucasian samples in the same study [18]. Besides, neurotransmitters like glutamate and GABA can also be affected by inflammation. Study on depressed adolescents found higher levels of pro-inflammatory cytokines are associated with higher levels of glutamate [19]. Increased body levels of glutamic acid could lead to hyperglutamatergic neurotransmission, which may result in the occurrence of depression [20]. Another study showed that patients with first episode depression had significantly decreased glutamate and increased GABA levels compared to healthy controls [21].

Serum lipids may be linked to depression via alteration of serotonin metabolism, the neurotransmitter in our brain that regulates our mood. Cholesterol has crucial role in brain development and in neuron-to-neuron signaling [22]. Past studies had shown that low-density lipoprotein (LDL) cholesterol levels are associated with depression [23–25]. LDL cholesterol can reduce availability of serotonin and increase depression risk [26] by directly impair the function of the serotonin 1A receptor in the brain. Activation of this receptor is associated with many other neurotransmitters related to recovery and repair of neuron, as well as depression [27]. Daut and Fonken [28] suggested that alterations in the serotonin system may disrupts circadian rhythms and increases a person's susceptibility to depression. A recent study found rapid response to selective serotonin reuptake inhibitors (SSRIs) in post-COVID depressed patients, their findings suggests that SSRIs could be an effective depression treatment option for the neuroinflammation triggered by SARS-CoV-2 [29]. However, there is a recent review opposes the idea that serotonin cause depression. The researchers found no consistent evidence showing association between serotonin and depression [30].

4. Conclusions

In short, most protein markers identified in this study were related to inflammation and lipid transport. Discovery and identification of easily accessible depression biomarker like serum proteins could enhance our understanding of the pathophysiology of depression, as well as providing possible treatment targets for depression. Future studies with healthy controls are much needed to further confirm the role of these biomarkers in depression. Future studies could also look into the relationship between these inflammation markers and dietary patterns, so that we can modify our food intake for the early prevention of depression.

Author Contributions: Conceptualization, C.N.F., Y.M.L., F.L.N. and P.Y.T.; methodology, C.N.F., Y.M.L., F.L.N. and S.Y.Y.; validation, C.N.F., Y.M.L., F.L.N., S.Y.Y., K.-S.P. and S.M.-S.; formal analysis, C.N.F. and S.Y.Y.; investigation, C.N.F., Y.M.L., F.L.N. and S.Y.Y.; data curation, S.Y.Y.; writing—original draft preparation, S.Y.Y.; writing—review and editing, C.N.F., Y.M.L., F.L.N., S.Y.Y., K.-S.P., S.M.-S., P.Y.T. and J.K.N.S.; supervision, C.N.F., Y.M.L., and F.L.N.; project administration, C.N.F., Y.M.L., F.L.N. and S.Y.Y.; funding acquisition, C.N.F., Y.M.L., F.L.N., P.Y.T., S.M.-S. and J.K.N.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the Universiti Tunku Abdul Rahman Research Fund (UTARRF), (Project number IPSR/RMC/UTARRF/2019-C2/F01 and IPSR/RMC/UTARRF/2022-C1/F02).

Institutional Review Board Statement: The study was conducted according to the Declaration of the UTAR Research Ethics and Code of Conduct guidelines, Code of Practice for Research Involving Humans, and approved by the UTAR Scientific and Ethical Review Committee (SERC) (U/SERC/194/2022).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank the students, without whom the research would not be possible.

Conflicts of Interest: The authors declare no conflict of interest. The funder had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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