Carotenoid as a Potential Antioxidant Marker for Schizophrenia
(Karotenoid Berpotensi sebagai Penanda Antioksidan bagi Skizofrenia)

T. J. CHOW & H.C. LOH

ABSTRACT
Free radicals are results of aerobic activities and can damage cells when present in excess by causing oxidative stress. Antioxidants efficiently quench free radicals to counteract oxidative stress. Carotenoids are antioxidants that have detectable natural colorant which can be measured as indicator of antioxidant level in human. The aim of this study is to investigate possible association of carotenoid antioxidant levels in schizophrenia. A total of 524 patients with schizophrenia from Hospital Bahagia Ulu Kinta, Malaysia and 391 healthy controls were recruited. Subjects’ skin carotenoid levels were measured through a non-invasive approach using Raman spectroscopy. Patients with schizophrenia showed significant (p < 0.01) lower carotenoid level compared to healthy controls. Factors such as gender, age, subtypes, antipsychotic drug treatments, and duration of illness did not differ significantly among patients. It is concluded that patient with schizophrenia have low levels of carotenoid antioxidants and is suggested to experience higher level of oxidative stress compared to healthy individuals.

Keywords: Antioxidant; carotenoid; oxidative stress

INTRODUCTION
Schizophrenia (SCZ) is a major psychiatric disorder that alters an individual’s perception, thought, mood and behavior (Malaysian Psychiatric Association 2009). It affects 1 % of the world population and each individual has 0.7 % risk of developing the disorder over lifetime (Bilder 2006; Tandon et al. 2008). There are variations in the incidence of SCZ, where family history, urban-living and oxidative stress are linked to a higher risk for developing the disorder (Othmen et al. 2008; Tsuboi et al. 2006).

Free radicals are reactive oxygen species reported to play a major role in the aetiology of many diseases such as diabetes (Webb & Falkowski 2009), neuropsychiatric disorder (Bilici et al. 2001), SCZ (Othmen et al. 2008) and many age-related diseases. They are usually produced as the body immune response to exogenous oxidants for instance, cigarette smoke, inappropriate diet and radiation (Packer 2006). Free radicals seek to damage cellular components such as lipid, which is essential in nerve cell membrane function and would affect signal transmissions and might degenerate brain functions (Block 1999).

Antioxidants serve to counteract oxidative stress, improving immune functions and prevent diseases (Surh & Packer 2005). Examples of antioxidants in human plasma and tissue are vitamin A, vitamin C, vitamin E, and the carotenoids (Nishino et al. 2002). Amongst them, carotenoids are unique in their natural colorants that are distributed in human tissue and can be detected as certain wavelengths when they are stimulated (Khachik et al. 1999). Carotenoids not only inhibit free radicals, they are also efficient quenchers of the cancer-causing singlet oxygen. Important sources of carotenoid include: red, orange, yellow, and green vegetable and fruits (Stahl & Sies 1999). Skin carotenoid has been reported as a
The sample population consisted of 524 SCZ subjects recruited from Ulu Kinta Psychiatric Hospital (HBUK), Perak, Malaysia and 391 healthy control subjects in the study period from February 2008 until December 2009. Socio-demographic data (age and gender) and other relevant clinical information (duration of illness, subtypes of SCZ and medications used) were collected. The 380 male and 144 female patients were grouped into their respective subtype: catatonic (n = 4), disorganised (n = 96), paranoid (n = 217), residual (n = 8), and undifferentiated (n = 199). The diagnoses of SCZ subjects were done according to the Mini International Neuropsychiatric Interview (M.I.N.I.), English version 5.0.0 (Sheehan et al. 1998).

The 391 healthy control subjects (male = 183, female = 208) were selected based on the following exclusion criteria: (1) no personal history of mental disorders, (2) no family history of mental disorders, (3) no history of major illness such as cancer and AIDS, (4) not on drug therapy or substance abuse and lastly (5) non-smoker. Dietary fruits and vegetables intakes of controls were ascertained. Only those who had two to three servings of fruits and vegetables intake per day, matching the diet of patients, were recruited in this study. Due to smoking restriction in HBUK, and population compatibility, only non-smoking controls were recruited.

Both patients and controls were residents of Malaysia, with age ranging from 16 to 80 years (patient mean age = 44.95 ± 12.50; control mean age = 32.82 ± 11.76). Written consents were obtained from subjects before participation in the study. This study was approved by the Medical Research & Ethics Committee (MREC), Ministry of Health (MoH), Malaysia (KKM/NIHSEC/08/0804/P07-42).

**MEASUREMENT OF CAROTENOID LEVEL**

Carotenoid levels of subjects were measured using Pharmanex® BioPhotonic Scanner S2 (Pharmanex, Provo, U.S.A.). Subjects were required to wash and dry their palms prior to the measurement and place the palm on the Scanner (in front of the Raman laser) for two minutes. Individual skin carotenoid was recorded and compiled for analysis. Score were categorised into five ranges: the lowest range below 20,000; followed by value ranging from 20,001 to 30,000; 30,001 to 40,000; 40,001 to 50,000; and lastly, the highest range of 50,001 to 60,000.

**VALIDATION BY FRAP ASSAY**

As a validation to the skin carotenoid level obtained using Raman spectroscopy, Ferric Reducing Ability of Plasma (FRAP) assay was performed on 87 patients and 70 controls that were randomly chosen from the main study group. This assay is based on the reduction of ferric (III) to ferrous (II) through antioxidants. Using a modified method adapted from Benzie and Strain (1996), total antioxidant level of the serum samples were determined using FRAP assay. Fresh FRAP solution were mixed with serum samples and incubated for 10 min in dark before measurement of absorbance at 593 nm.

**STATISTICAL ANALYSIS**

Data analyses were perform using Statistical Package for Social Sciences (SPSS) version 16.0 for Windows (SPSS Inc, Chicago, USA). Carotenoid score differences between SCZ and control subjects were evaluated using
independent sample T-test. Variables tested using one-way analysis of variance (ANOVA) included gender, age, SCZ subtypes, patient medication, depot injection and duration of illness.

In the analysis of SCZ subtype, subjects with catatonic and residual subtype were excluded from the analysis due to low sample size. Both controls and patients were grouped into four age ranges: 16 to 30 years, 31 to 40 years, 41 to 50 years and more than 50 years of age. For the analysis of medication consumption, patients were divided into three categories depending on their medication: typical antipsychotics such as haloperidol and chlorpromazine; atypical antipsychotics such as olanzapine and clozapine; and other psychotropics that are not in the above two categories, such as diazepam and fluvoxamine. Further analysis of depot injection compared patients that received intramuscular injection of antipsychotics with patients that did not receive any injections. In the analysis of duration of illness, patients were grouped into four duration periods: ill for less than 5 years, between 5 and 10 years, between 10 and 20 years and above 20 years.

For each studied population, Levene’s homogeneity test was used to test for equality variances. Descriptive statistics were calculated for all variables and mean score and standard deviation (SD) was reported separately for each variable. \( p \)-value of < 0.01 was considered as significant.

The correlation between carotenoid level measured by FRAP assay and Raman spectroscopy was evaluated using Pearson’s correlation test, where \( r \)-value nearer to 1 was considered as a significant correlation.

## RESULTS

Each population in our study was homogenous for the equality of variances. Among the SCZ patients, 84 % obtained carotenoid scores less than 30,000. In contrast, 33 % of controls scored higher than 30,000. Significant (\( p < 0.01 \)) lower carotenoid score was observed in patients compared to controls. Mean carotenoid score of controls and patients were 27,200 ± 7675 and 22,213 ± 8774 respectively, showing 18% difference. On average, female obtained significantly (\( p < 0.01 \)) higher mean carotenoid score compared to male (Table 1). Mean scores for females in the control and patient group were 15 % and 17 % significantly (\( P < 0.01 \)) higher than that of males respectively.

The mean carotenoid scores with respect to different age ranges are summarised in Table 1. We studied mean carotenoid score between patients and controls (1) within individual age range and (2) between different age ranges. Comparison between controls and patients in different age range showed no statistical significance, but on average, controls’ mean score were 14.7 % higher than patients.

In subtype analysis (Figure 1), carotenoid levels for disorganised (mean = 22,106 ± 6975), paranoid (mean = 21,715 ± 7984), and undifferentiated (mean = 22,585 ± 7735) subtypes were not significantly different from each other. On the other hand, mean carotenoid score of patients with atypical antipsychotics treatment were found to be higher (mean = 23,045 ± 7727) compared to those typical antipsychotics treatment (mean = 22,119 ± 7898) and other psychotropic medications (mean = 21,430 ± 7098). ANOVA did not show significant difference in mean score and medications among patients. Similarly, depot injection and duration of illness did not show significant difference with carotenoid score.

For the FRAP validation assay, the correlation between carotenoid level evaluated by FRAP assay and Raman spectroscopy measurement was determined. The relationship was established as equation of \( y = 0.024x \) (\( R^2 = 0.688 \)) for controls (Figure 2), and \( y = 0.027x \) (\( R^2 = 0.562 \)) for patients (Figure 3). Analysis of Pearson’s correlation showed significant strong positive correlation between FRAP antioxidant level and skin carotenoid level in controls (\( r = 0.830, p < 0.01 \)) and patients (\( r = 0.811, p < 0.01 \)).

### TABLE 1. Mean carotenoid score for demographic characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Mean Carotenoid Score ± SD</th>
<th>( n )</th>
<th>( P )-value</th>
</tr>
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<tbody>
<tr>
<td>Gender</td>
<td></td>
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<tr>
<td>Male</td>
<td>25.257 ± 8571</td>
<td>183</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Female</td>
<td>28.865 ± 8814</td>
<td>208</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>( P )-value</td>
<td></td>
<td></td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-30 years</td>
<td>25.406 ± 7286</td>
<td>209</td>
<td>&gt; 0.01</td>
</tr>
<tr>
<td>31-40 years</td>
<td>28.418 ± 8322</td>
<td>70</td>
<td>&gt; 0.01</td>
</tr>
<tr>
<td>41-50 years</td>
<td>28.706 ± 9882</td>
<td>54</td>
<td>&gt; 0.01</td>
</tr>
<tr>
<td>&gt;50 years</td>
<td>29.285 ± 1,1245</td>
<td>49</td>
<td>&gt; 0.01</td>
</tr>
</tbody>
</table>

- Mean carotenoid score was 27,200 ± 7675 for controls and 22,213 ± 8774 for patients.
- Female patients had a higher mean carotenoid score compared to male (\( p < 0.01 \)).
- Controls and patients had no significant difference in mean score for different age ranges.
- In subtype analysis, disorganised, paranoid, and undifferentiated subtypes had no significant difference. Atypical antipsychotics treatment showed a higher mean score compared to typical antipsychotics and other psychotropics.
- FRAP validation assay showed a strong positive correlation between carotenoid level and carotenoid level evaluated by Raman spectroscopy.
FIGURE 1. Mean carotenoid score for clinical characteristics of patients. Number of patients ($n$) is stated in brackets.

FIGURE 2. Relationship between FRAP antioxidant level with control skin carotenoid level.

FIGURE 3. Relationship between FRAP antioxidant level with patient skin carotenoid level.

$y = 0.0238x$

$R^2 = 0.6883$

$y = 0.0271x$

$R^2 = 0.5618$
DISCUSSION

The total antioxidant level evaluated by FRAP assay correlates with carotenoid level measured by Raman spectroscopy. Results showed the trend of increasing serum antioxidant level with increasing skin carotenoid level. This positive correlation trend emphasised that carotenoid correspond to the total antioxidant capacity human serum (Svilaas et al. 2004).

The main findings of the present study are that (1) SCZ was characterised by significantly ($p < 0.01$) lower carotenoid level compared to controls; and (2) the possibility of treatment with antipsychotics as the main factor for the reduced antioxidant levels shown in patients. Recent studies on plasma antioxidant in SCZ patients showed similar trend of reduced antioxidant level that is associated to high oxidative stress (Kunz et al. 2008; Reddy et al. 2003). However, it is important to investigate whether this trend applies solely to certain SCZ subtypes. Gama et al. (2008) reported no effect of different subtypes on antioxidant level. Similarly, our study observed that carotenoid level did not significantly associate with subtypes in the patient group, and thus suggested that subtype differentiation has no or equal effect on oxidative damage.

Both control and patient female have significantly higher carotenoid score than males suggesting that gender is not an affecting factor in SCZ. Rather, the association between plasma antioxidant and hormone prolactin in SCZ suggested that sex differences in oxidative stress may be related to hormone differences between males and females (Zhang et al. 2006).

Aging and age-related diseases are often associated with high levels of free radicals and oxidative stress. An elderly person would have lower antioxidant level and more susceptible to diseases compared to a younger person (Buijsse et al. 2005). In the process of brain aging, free radicals attack the lipid composition of brain cell membrane, resulting in abnormal signal transduction that is portrayed in symptoms of mental illness (Mazza et al. 2007). This trend applies in the SCZ patient group, but not the control group, where the oldest control group have higher score the youngest control group. Overall results suggest SCZ might contribute to the low carotenoid level in patients. On the other hand, analysis in duration of illness did not differ significantly among patients, thus was excluded as contributing factor of reduced antioxidant level.

Antipsychotic drugs serve to relieve schizophrenic symptoms but they would also produce distressing extrapyramidal side effects and some patients may develop tardive dyskinesia (Liska 2004; Parrott et al. 2004). Tardive dyskinesia is associated with low antioxidant levels in smokers, suggesting possible relationship between antioxidant and antipsychotic drugs (Dielh et al. 2009). Treatments with antipsychotic drugs induce lipid peroxidation and oxidative stress, thus lowering antioxidant level (Schmidt et al. 2008). Similarly, our findings showed that regardless of the type of antipsychotics, patients under drug treatment have a significant lower antioxidant score compared to controls. Intramuscular depot injection allows direct entry of antipsychotics into the bloodstream which promotes immediate effect of the drug but this might also cause more serious side effects (Liska 2004). Our results indicated that carotenoid score of patients with and without injection were not statistically different, suggesting depot injection does not further increase oxidative stress in patients. Gender, age, and duration of illness were thus eliminated as factors reducing carotenoid level.

In conclusion, SCZ patients have significantly lower carotenoid level compared to healthy controls, indicating higher level of oxidative stress in SCZ. Antipsychotic treatment and SCZ illness were suggested to be the possible reasons to the reduction of antioxidant level in schizophrenic patients. The strength of this paper is the sample size is good and it is among one of the first research into measuring carotenoid level of people suffering from SCZ using a non-invasive method. The limitation of this current study is that we did not study the lifestyle and diet as factors reducing carotenoid level. Further study that includes dietary antioxidant supplement in treatment, ethnic and antipsychotic drug dosage may be rewarding.

ACKNOWLEDGEMENTS

This research was supported by Universiti Tunku Abdul Rahman (UTAR, Research Grants 6200/L02 and 6202/C01). We are very grateful to Dato’ Dr. Suarn Singh Jasmit Singh, Director of Ulu Kinta Hospital Bahagia and also all other psychiatrists (Yee Chuang Cheah, Rabaiah Mohd Salleh, Tak Wah Loo, Razifah Abdul Rahman, Satnam Kaur Harbhajan Singh, Zulkifri Ghaus, Ahmad Syukri Chew Abdullah, Bilbhir Kaur Chigara Singh) who participated in patient diagnosis and blood sample collection.

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Received: 12 January 2011
Accepted: 30 December 2011