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Carotenoid as a Potential Antioxidant Marker for Schizophrenia

(Karotenoid Berpotensi sebagai Penanda Antioksidan bagi Skizofrenia)

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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

ABSTRAK

Radikal bebas merupakan hasilan daripada aktiviti aerobick dan boleh merosakkan sel melalui tekanan oksidatif apabila hadir secara berlebihan. Antioksidan menghilangkan radikal bebas untuk mengatasi tekanan oksidatif secara berkesan. Antioksidan karotenoid adalah pewarna semula jadi yang dapat dikesan serta diukur sebagai penanda aras antioksida dalam manusia. Matlamat kajian ini adalah untuk menyiasat kemungkinan wujudnya hubungan paras antioksida karotenoid dalam skizofrenia. Sejumlah 524 pesakit skizofrenia dari Hospital Bahagia Ulu Kinta, Malaysia dan 391 kawalan yang sihat direkrut. Aras karotenoid di kulit subjek diukur melalui spektroskopi Raman yang "tidak-invasif". Pesakit skizofrenia menunjukkan paras karotenoid yang rendah secara nyata ($P > 0.01$) berbanding dengan kawalan sihat. Faktor-faktor seperti jantina, umur, sub-jenis, rawatan ubat antipsikotik dan tempoh sakit adalah tidak signifikan di kalangan pesakit. Secara kesimpulannya, pesakit skizofrenia mempunyai aras antioksidan karotenoid yang lebih rendah dan dicadangkan mengalami paras tekanan oksidatif yang lebih tinggi berbanding dengan individu yang sihat.

Kata kunci: Antioksidan; karotenoid; tekanan oksidatif

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

reliable indicator of overall antioxidant level in human that provides evidence of oxidative stress which is often related to diseases (Svilaas et al. 2004).

Factors such as lifestyle, dietary habit and smoking habit affect individual antioxidant level. Deteriorating lifestyle factors such as excessive emotional stress, alcohol and drug abuse induce free radicals production in human brain (Hiramatsu 2006; Tsuboi et al. 2006). Diet heavy on fats with considerable less fruits and vegetables would result in low antioxidant level due the presence of polyunsaturated fats in most fried and overcooked food that would react with free radicals in a chain reaction, exhausting the antioxidants and intensifying oxidative damage (Hiramatsu 2006). Similarly, antioxidant level in blood and tissue of smokers tends to be lower than non-smokers. Combustion of the tar component in cigarettes actively reduced oxygen to hydrogen peroxide, causing oxidative stress (Duthie 1999). Smoking psychiatric patients develop more psychotic symptoms and require higher dose of antipsychotics compared to non-smoking patients (Diehl et al. 2009).

Most antioxidant studies on diseases were done by measuring enzyme activity in red blood cell and plasma using high-performance liquid chromatography techniques. However, such methods are invasive, expensive and impractical for a large sample size. In the present study, total antioxidant level was determined by a simple, rapid, and non-invasive technique using Raman spectroscopy which utilises laser spectroscopic technique to detect characteristic vibration energy of carotenoids on human skin (Hata et al. 2000). Although Othmen et al. (2008) argued that peripheral markers may not reflect similar changes in the central nervous system, research demonstrated a significant correlation between serum carotenoid and skin carotenoid levels (Zidichouski et al. 2004), attributing the Raman spectroscopy method to be more specific, sensitive, cost effective and reproducible. Thus, antioxidant detection using Raman spectroscopy has been applied in illnesses such as cancer, cardiovascular disease (Smidt 2005) and eye disease (Zhao et al. 2003). This study examined possible differences of skin carotenoid level in relation to demographic and clinical characteristics between SCZ and healthy population.

Materials and Methods

Subject Selection

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

Characteristics	Mean Carotenoid Score ± SD				
	n	Control	*n*	Patient	*P*-value
Gender					
Male	183	25,257 ± 8571	380	21,540 ± 7434	< 0.01
Female	208	28,865 ± 8814	144	23,990 ± 8030	< 0.01
P-value		< 0.01		< 0.01	
Age					
16-30 years	209	25,406 ± 7286	65	23,364 ± 9045	> 0.01
31-40 years	70	28,418 ± 8322	147	22,662 ± 8067	> 0.01
41-50 years	54	28,706 ± 9882	144	22,402 ± 7324	> 0.01
>50 years	49	29,285 ± 1,1245	178	22,247 ± 7965	> 0.01

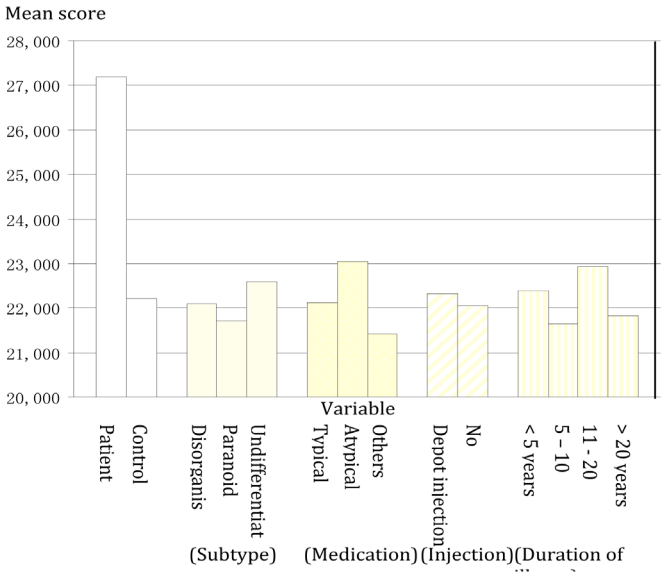

FIGURE 1. Mean carotenoid score for clinical characteristics of patients. Number of patients (*n*) is stated in brackets

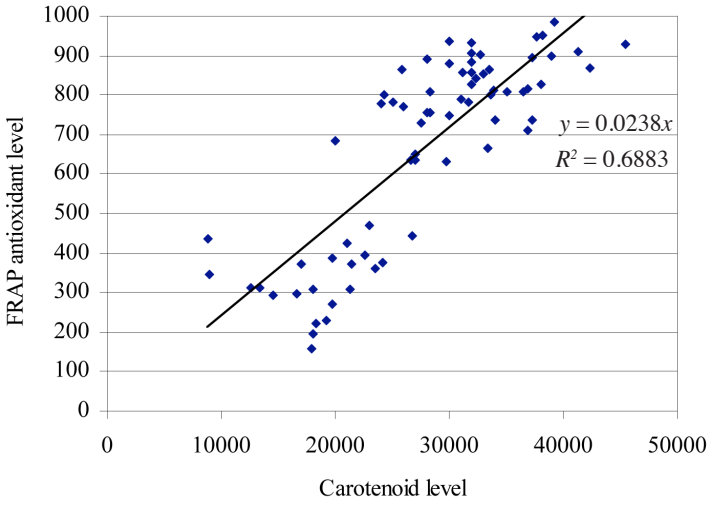

FIGURE 2. Relationship between FRAP antioxidant level with control skin carotenoid level

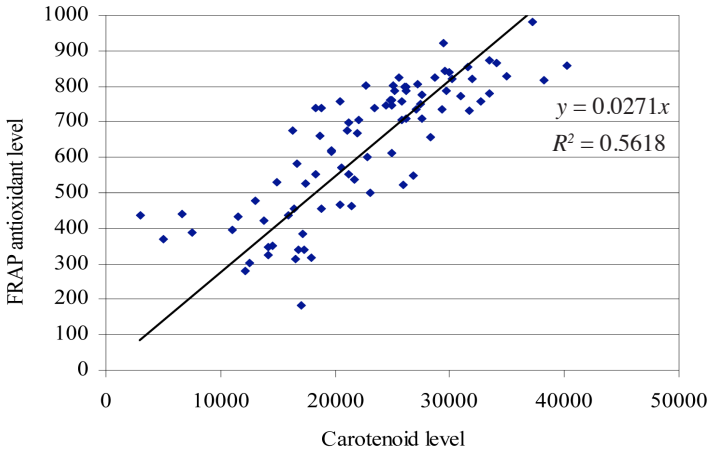

FIGURE 3. Relationship between FRAP antioxidant level with patient skin carotenoid level

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 (Diehl 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.

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References

Bilder, R.M. 2006. Schizophrenia, In, *Clinical Neuropsychology: A Pocket Handbook For Assessment* pp. 398-414. Snyder, P.J., Nussbum, P.D. & Robins, D.L. (eds.) Washington: American Psychological Association.

Block, G. 1999. Emerging role of nutrition in chronic disease prevention: A look at the data, with an emphasis on vitamin C, In, *Antioxidant Food Supplements in Human Health*. pp. 45-49. Packer, L., Hiramatsu, M. & Yoshikawa, T. (eds). San Diego: Academic Press.

Buijsse, B., Feskens, E.J.M., Schlettwein-Gsell, D., Ferry, M., Kok, F.J. & Kromhout, D. 2005. Plasma carotene and α-tocopherol in relation to 10-y all-cause and cause-specific mortality in European elderly: the survey in Europe on Nutrition and the elderly, a concerted action (SENECA). *Am. J. Clin. Nutr.* 82: 879-886.

Bilici, M., Efe, H., Koroglu, M.A., Uydu, H.A., Bekaroglu, M. & Deger, O. 2001. Antioxidative enzyme activities and lipid peroxidation in major depression: alterations by antidepressant treatmeants. *J. Affect. Dis.*64: 43-51.

Diehl, A., Reinhard, I., Schmitt, A., Mann, K. & Gattaz, W.F. 2009. Does the degree of smoking effect the severity

of tardive dyskinesia? A longitudinal clinical trial. *Eur. Psychiatry* 24: 33-40.

Duthie, G.G. 1999. Natural antioxidant in the protection against cigarette smoke injury. In, *Antioxidant food supplements in human health*. Packer, L., Hiramatsu, M. & Yoshikawa, T. (eds.) pp. 35-42. San Diego: Academic Press.

Gama, C.S., Salvador, M., Andreazza, A.C., Lobato, M.I., Berk, M., Kapczinski, F. & Belmonte-de-Abreu, P.S. 2008. Elevated serum thiobarbituric acid reactive substance in clinically symptomatic schizophrenic patients. *Neurosci. Lett*. 433: 270-273.

Hata, T.R., Scholz, T.A., Ermakov, I.V., McClane, R.W., Khachik, F., Gellermann, W. & Pershing, L.K. 2000. Non-invasive Raman spectroscopic detection of carotenoids in human skin. *J. Invest. Dermatology* 115: 441-448.

Hiramatsu, M. 2006. Free radical scavengers and neuroprotection. In. *Molecular Interventions in Lifestyle-Related Diseases* Hiramatsu, M., Yoshikawa, T. & Packer, L. (eds.) Boca Raton: CRC Press. pp. 11-23.

Khachik, F., Bertram, J.S., Huang, M.T., Fahey, J.W. & Talalay, P. 1999. Dietary carotenoids and their metabolites as potentially useful chemoprotective agents against cancer. In, edited by *Antioxidant food supplements in human health*. Packer, L., Hiramatsu, M. & Yoshikawa, T. (eds.) San Diego: Academic Press. pp. 203-229.

Kunz, M., Gama, C.S., Andreazza, A.C., Salvador, M., Cereser, K.M., Gomes, F.A., Belmonte-de-Abreu, P.S., Berk, M. & Kapczinski, F. 2008. Elevated serum superoxide dismutase and thiobarbituric acid reactive substances in different phases of bipolar disorder and in schizophrenia. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry* 32: 1677-1683.

Liska, K. (ed.) 2004. *Drugs and the Human Body with Implications for Society*. New Jersey: Pearson Prentice Hall.

Malaysian Psychiatric Association. 2009. *Academy of Medicine Malaysia Clinical Practice Guidelines: Management of Schizophrenia in Adults*. Malaysia: Ministry of Health.

Mazza, M., Pomponi, M., Janiri, L., Bria, P. & Mazza, S. 2007. Omega-3 fatty acids and antioxidants in neurological and psychiatric diseases: An overview. *Prog. Neuro-Psychophamacol. Biol. Psychiatry* 31: 12-26.

Nishino, H., Murakoshi, M., Ii, T., Takemura, M., Kuchide, M., Kanazawa, M., Mou, X.Y., Wad, S., Masuda, M., Ohsaka, Y., Yogosawa, S., Satomi, Y. & Jinno, K. 2002. Carotenoids in cancer chemoprevention. *Cancer Metastasis Rev.* 21: 257-264.

Othmen, L.B., Mechri, A., Fendri, C., Bost, M., Chazot, G., Gaha, L., & Kerkeni, A. 2008. Altered antioxidant defense system in clinically stable patients with schizophrenia and their unaffected siblings. *Prog. Neuro-Psychopharmacol. Biol. Psychiat*. 32: 155-159.

Packer, L. 2006. The antioxidant evolution: From free radical scavenging to the antioxidant network and gene regulation by flavonoid-rich extracts from pine bark and Gingko Biloba leaf, In, *Molecular Interventions in Lifestyle-Related Diseases* Hiramatsu, M., Yoshikawa, T. & Packer, L. B(eds.) Boca Raton: CRC Press, pp. 205-208.

Parrott, A., Morinan, A., Moss, M. & Scholey, A. 2004. *Understanding Drugs and Behaviour*. England: John Wiley & Sons Ltd.

Reddy, R., Keshavan, M. & Yao, J.K. 2003. Reduced plasma antioxidants in first-episode patients with schizophrenia. *Schizophr. Res*. 62: 205-212.

Schmidt, A.J., Hemmeter, U.M., Krieg, J., Vedder, H. & Heiser, P. 2008. Impact of haloperidol and quetiapine on the expression of genes encoding antioxidant enzymes in human neuroblastoma SH-SY5Y cells. *J. Psychiatr. Res*. 43: 818-823.

Sheehan, D., Lecrubier, Y., Janavs, J., Baker, R., Sheehan, K.H., Knapp, E., Sheehan, M., Weiller, E., Hergueta, T., Amorim, P., Bonora, L.I. & Lepine, J.P. 1998. *M.I.N.I. Mini International Neuropsychiatric Interview, English version 5.0.0, DSM-IV*. Tampa: University of South Florida.

Smidt, C.R. 2005. Non-invasive Raman spectroscopic detection of carotenoids in human skin as a biomarker of antioxidant status. *J. Korean Acad. Fam. Med*. 26: 398-408.

Stahl, W. & Sies, H. 1999. Carotenoids: Occurrence, biochemical activities, and bioavailability. In, *Antioxidant Food Supplements in Human Health*. Packer, L., Hiramatsu, M. & Yoshikawa, T. (eds.) San Diego: Academic Press, pp. 183-196.

Surh, Y.J. & Packer, L. 2005. *Oxidative Stress, Inflammation, and Health*. USA: Taylor and Francis.

Svilaas, A., Sakhi, A.K., Andersen, L.F., Svilaas, T., Strom, E.C., Jacobs, D.R., Ose, L. & Blomhoff, R. 2004. Intakes of antioxidants in coffee, wine, and vegetables are correlated with plasma carotenoids in humans. *J. Nutr.* 134: 562-567.

Tandon, R., Keshavan, M.S. & Nasrallah, H.A. 2008. Schizophrenia, "Just the facts" What we know in 2008, Epidemiology and etiology. *Schizophr. Res*. 102: 1-18.

Tsuboi, H., Tatsumi, A., Yamamoto, K., Kobayashi, F., Shimoi, K. & Kinae, N. 2006. Possible connections among job stress, depressive symptoms, lipid modulation and antioxidants. *J. Affect. Dis*. 91: 63-70.

Webb, C.B. & Falkowski, L. 2009. Oxidative stress and innate immunity in feline patients with diabetes mellitus: the role of nutrition. *J. Feline Med. Surg*. 11: 271-276.

Zhao, D.Y., Wintch, S.W., Ermakov, I.V., Gellermann, W. & Berstein, P.S. 2003. Resonance Raman measurement of macular carotenoids in retinal, choroidal, and macular dystrophies. *Arch. Ophthalmol*. 121: 967-972.

Zhang, X.Y., Tan, Y.L., Cao, L.Y., Wu, G.Y., Xu, Q., Shen, Y. & Zhou, D.F., 2006. Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. *Schizophr. Res*. 81: 291-300.

Zidichouski, J.A., Poole, S.J., Gellermann, W., Smidt, C.R. 2004. Clinical validation of a novel Raman spectroscopic technology to non-invasively assess carotenoid status in humans. *J. Am. College Nutr.* 23: 468.

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