Original article

D4 and D5 dopamine receptor mRNA expression in human peripheral blood lymphocytes among co-occurring opioid and amphetamine-type stimulants use disorder undergoing methadone maintenance therapy

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Abstract

Introduction: Opioid and amphetamine type stimulant (ATS) exert their rewarding effects by stimulating the dopaminergic system in the mesolimbic area. It has been suggested that dopamine system in peripheral blood lymphocytes reflect the central dopamine system's activity and pathology, especially in neuropsychiatric diseases including drug addiction. The present study aimed to assess the effect of co-occurring opioid and ATS (COATS) addiction towards mRNA expression of dopamine receptors DRD4 and DRD5 in peripheral blood lymphocytes (PBLs) of drug dependent subjects (n=36) undergoing methadone maintenance therapy in comparison to control subjects (n=36). ***Materials and methods*:** Ten mL blood were obtained from the subjects followed by lymphocyte isolation, RNA extraction and reverse transcription. DRD4 and DRD5 mRNA expression in peripheral lymphocytes was assessed using real-time PCR. The DRD4 mRNA expression but not DRD5 was significantly reduced in the peripheral lymphocytes of COATS subjects. ***Results:*** Mean expression value for DRD4 was 14.0±0.24 among patients and 13.3±0.25 among control subjects. For DRD5 it was 12.87 ± 0.75 among patients and 12.59 ± 1.24 among controls. ***Conclusion*:** Inconclusion, co-occurring opioid and ATS addiction was associated with persistent deficiency of DRD4 but not DRD5 in PBLs.

Keywords: Opioid; Amphetamine Type Stimulant (ATS); DRD4; DRD5; Methadone Maintenace Therapy (MMT)

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Introduction

Co-occurring opioid and amphetamine type stimulants (COATS) use is highly prevalent inflicting in major health problem world-wide[1,2]. Recent surveys in Malaysia among people who inject opioids revealed that 75% reported lifetime ATS use with 21% injecting. Lifetime ATS use was linked with HIV infection[3]. In Malaysia, popular ATS include crystalline methamphetamine and amphetamine/methamphetamine tablets while heroin and morphine remain the most commonly misused opioid[4]. Methadone maintenance treatment (MMT) is

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a comprehensive and effective substitution treatment for opioid use disorder and was commenced in Malaysia in 2006 by Malaysia Ministry of Health[5,6]. In comparison to opioid, methadone has fewer side effects, improves mental and physical well-being and reduces mortality[7, 8, 9.]

Dopaminergic system in the brain reward centrelocated in the mesolimbic area serves as the major target in drug addiction[10],[11]. Opioids and ATS hijack this brain area by altering the natural dopamine neurotransmission leading to extracellular hyper-dopamine state resulting in 'high' state[11].Dopamine actions are mediated by five G protein-coupled receptor subtypes, D1-like (the D1 and D5) receptor which reduced the reward-seeking motivation, while the activation of D2-like (the D2, D3 and D4) receptors is associated with reinforcement[12]. The association of D1, D2 and D3 dopamine receptor and addiction has been studied widely while the role of dopamine D4 and D5 receptor remained scarce.

Human peripheral blood lymphocytes (PBLs) have been reported to indicate dopamine receptors of D3, D4 and D5.It has been hypothesized that any changes in dopamine receptors expression level in PBL reflected brain's dopamine status[13,14]. In agreement, Nagai *et al*., (1996)[15] reported that reduction of striatal dopaminergic neurotransmission in Parkinson disease was associated with reduction of PBL dopamine expression that correlated with clinical severity.Czermak *et al*., (2004)[16] observed that the reduced PBL D4 mRNA expression in long-term abstinent alcohol and heroin addicts, suggesting for persistent dopamine imbalance in abstinent addicts. Goodarzi *et al*., (2009)[17] also suggested that persistent dopamine D4 and D5 receptors' deficiency contributed to the risk for addiction as they found that PBL D4 and D5 receptor expression were significantly reduced in abstinent subjects. However, information about the D1 and D2 expression in PBL were inconsistent [17],[18].

Hence, this study aimed to assessDRD4 and DRD5 mRNA expression in COATS patients undergoing MMT.PBL may represent promising tool to investigate the central dopamine pathologies as well as to monitor the efficiency of pharmacologic and therapeutic intervention in addicted individuals[19].

MATERIAL AND METHODS

Subjects

Seventy-two Malay males were enrolled. Thirty-six were patients with opioid and ATS (COATS) use disorder undergoing MMT at a Health Clinic in Kuala Terengganu with the remaining serving as control subjects. Inclusion criteria were as follows: (1) Diagnosis for COATS based on DSM-5(2) male, aged from 18 to 50 years; (3) no previous history or current mental disorder including major depression, psychoses, or bipolar disorder; (4) seronegative for human immunodeficiency virus (HIV); and (5) Malay (third generation). Background information of patients, including name, age, education level, marital status, occupation, and substance abuse history, were included in data collection. Blood was collected during the no-opioid withdrawal period. The study was performed at Bukit Tunggal Health Clinic in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines and subjects gave voluntary informed consents.

Blood collection and RNA extraction

Ten mL venous blood was collected from the antecubital vein into a sterile lithium heparin tube (BD VACUTAINER® Blood Collection Tubes) and processed within two hours. Peripheral blood lymphocytes (PBLs) were isolated using lymphocyte separation media (Lymphoprep, Axis Sheild) density centrifugation, and transferred into 1.5mL microcentrifuge tubes (RNAse free) and stored at -80°C until use. Total RNA extraction was performed with QIAamp RNA Blood Mini Kit according to the protocols of the manufacturer (QIAGEN, GmbH, Germany). RNA concentration and purity were measured using the NanoPhotometer Np80 (Implen, Germany) system. Acceptable ratios of optical densities (OD) at 260nm and 280nm were in the range of 1.8–2.0. QuantiNova™ Reverse Transcription Kit (QIAGEN GmbH, Germany) was used for cDNA synthesis of purified total RNA.

Expression of mRNA DRD4 and DRD5 genes by Real-Time PCR

DRD4 and DRD5 mRNA expression was assessed using the Step OnePlus Real-Time PCR System (Applied Biosystems) and a commercial SYBR Green RT-PCR kit (Qiagen, Hilden, Germany). Specific primers for DRD4 and DRD5 and β-actin that was used as a reference gene were purchased from company primer bank (MyTACG Bioscience Enterprise, Malaysia). The following primers were used. DRD4: forward primer, 5'-CTGCCGCTCTTCGTCTACTC-3'; reverse

primer, 5' -ATGGCGCACAGGTTGAAGAT-3'. DRD5: forward primer, 5'-CTCTTCTCTCGCTCCGAACC-3'; reverse primer, 5' -TCTCTTGCCTCCTGAAGCG-3'. β-actin forward primer, 5'-CCGGCCAGCCAGGTCCAGA-3'; reverse primer, 5' - CAAGGCCAACCGCGAGAAGATG-3'(Ostadali *et al*., 2004; Latheef *et al*., 2016). RT-PCR amplification was performed with an initial PCR activation step at 95°C for 2 min, followed by 40 cycles of 2 step-cycling; denaturation at 95°C for 5 sec and combined annealing/extension at 60°C for 30 sec, and then held at 95°C for 5 sec. The single peak in melt curve analysis was used to assess the specificity of PCR products. Standard curves were constructed to assess the real-time PCR assay's linearity by using serial dilutions of PBL cDNA at 1:2, and regression coefficients (r2) were always >0.900. Expression data were acquired from threshold cycle (CT) values. The difference in CT values for each target gene was calculated as follows: ΔCT = mean $CT_{(target\ gene)}$–$CT_{(reference\ gene).}$

Data analysis

Continuous variables were expressed as mean ± standard deviation (SD). Differences were assessed using independent t-test and Mann–Whitney test. All analyses were performed using Graph Pad Prism (Graph Pad Prism, version 6 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Ethical Clearance: The protocols for this study were approved by the Medical Research and Ethics Committee (MREC), Ministry of Health Malaysia (NMRR-18-1989-41507 (IIR)).

Results

Average age of patients recruited was 41 years (40.94±4.64), controls 23 years (26.81±8.47). The methadone daily doses averaged 65.0mg(65.0±26.3), treatment duration 5 years (5.23±2.31), and age of the first-time drug abuse 23 years old(23.23±4.301). As shown in Table 1, the DRD4 mRNA expression level was significantly reduced among COATS patients compared to healthy controls. However, the difference in the DRD5 mRNA expression level did not reach statistical significance. Figure A and Figure B showed normalized CT (ΔCT) value of DRD4 and DRD5 mRNA expression by real-time PCR from drug dependent subjects (n=36) and control subjects (n=36) respectively.

Table 1: Mean normalized expression values (ΔCt) of ATS and opioid disorder patients and healthy controls.

	N	Mean Normalized expression Values (ΔCt) (SEM)	p-value
DRD4			
Patients	36	14.00 ± 0.24	0.0393*[a]
Healthy Control	36	13.30 ± 0.25	-
DRD5			
Patients	36	12.87 ± 0.75	0.2511[b]
Healthy Control	36	12.59 ± 1.24	

*There is a significant difference as p-value <0.05sig. (2-tailed) Mann Whitney-test Independentt- test

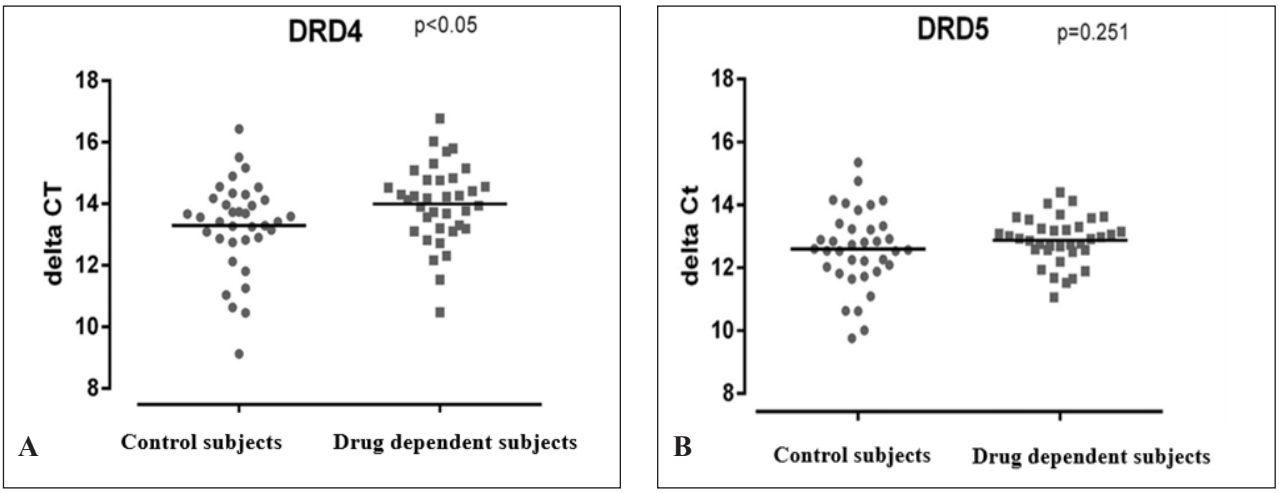

Figure A-B. Normalized CT (ΔCT) DRD5 mRNA expression by Real-Time PCR from drug dependent subjects (n=36) and control subjects (n=36). The line represents mean value.

DISCUSSION

Our study found a significantly reduced DRD4 in PBL mRNA expression but not in DRD5 of COATS undergoing MMT. Most abused drugs exert their effects by increasing the extracellular dopamine level in the nucleus accumbent in mesolimbic area giving such rewarding effects[22,11]. Changes in mRNA level of dopamine receptors in PBLs may reflect the central dopamine status and our finding reports the changes[11,23].

Study by Czermak *et al*., (2004)[16] suggested that the persistent deficiency of DRD4 increase the susceptibility of individuals to become addicted. Goodarzi *et al*., (2009)[17], reported a significant reduction of DRD4 expression in abstinent opioid and addicted subjects whereas the DRD4 level in opioid addicts undergoing MMT was not significantly reduced. In contrast, the findings of the present study demonstrated significant reduction of the dopamine D4 receptor's mRNA expression among COATS patients undergoing MMT. It is possible that the differences in these results are due to different effects of the abused drug in terms of neurobiological mechanisms and pathophysiology as the present study involved co-occurring opioid and ATS addiction. In addition, specific effect of stimulant drugs towards DRD4 may contribute to the findings of the present study. Self *et al* (1996)[24] reported that stimulant drug-seeking behaviour in rats was selectively induced and mediated by D2-like (D2, D3 and D4) dopamine receptor agonist subtype, and not by D1-like (D1 and D5) receptors subtype. Besides, the anatomical distribution and physiological properties of DRD4 upon DRD4 stimulation, leads to behavioural arousal and cognition improvement; property that confer with stimulant action hence make it important target of stimulant drugs[25].

Nevertheless, the persistent deficiency of DRD4 observed here suggested the involvement of DRD4 in long-lasting dysfunction of dopaminergic systems in drug dependent individuals. It is interesting to note that in major depression, DRD4 expression in PBL was found significantly reduced[26]. Indeed there occurs a high comorbidity of neuropsychiatric disorder and drug dependence as both are associated with mesolimbic dopaminergic deficiency[27]. Our results lend support to this as regards peripheral DRD4 expression. Taken together these findings suggest the possibility of using PBL DRD4 expression to serve as a biological marker in neuropsychiatric diseases.

It has been suggested that DRD5 mRNA expression in PBL may serve as peripheral marker in neurological and neuropsychiatric diseases. Barbanti *et al*., (1996)[28] found that DRD5 is the most well-defined dopamine receptors in lymphocyte characterised by both molecular biology and radio-ligand binding technique in migraine disease. Vousooghi *et al*. (2015)[14] reported a significant reduction of PBL DRD5 among computer game addicts. Our present study however showed no significant difference in the dopamine D5 receptor's mRNA expression in drug dependent subjects undergoing MMT. Goodarzi *et al*., (2009)[29] also reported that DRD5 expression level was not reduced compared to control in opioid addict patient undergoing MMT although they reported that the expression of DRD5 in long-term abstinent subjects was significantly reduced. The possible explanation of our findings is that the effect of MMT may assist in normalizing the dopamine D5 receptor expression as suggested by Allouche *et al,* (2015)[30]. Besides, DRD5 which belongs to D1-like receptors subtype hence DRD5 di not involved in stimulant drugs reinforcement effects[24]. In conclusion co-occurring opioid and ATS addiction was associated in persistent deficiency of DRD4 but not DRD5 in PBLs.

In conclusion, our findings suggest the possibility of using DRD4 expression in PBL as a peripheral biological marker in neuropsychiatric diseases including addiction. Further work is however required to define its role as a marker in drug addiction studies among COATS dependent individuals.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

Authors' contributions: All authors have same contributions in this above study.

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