Daily Clinical Dose (D) Poorly Predicts Trough Serum Methadone Concentration (C_{trough}) In Patients Undergo Methadone Maintenance Therapy (MMT) With Good Adherence

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ABSTRACT

Methadone maintenance therapy (MMT) has been used for opioid dependence as one of the harm-reduction approaches. It is also effectiveindirectlyto prevent the wide spread of HIV-related risks such as needle sharing behaviour and illicit drug use. With low costs calculated per patient, MMT has been put as top priorities on reducing opioid dependencies in Malaysia. However, the effective dose strategy of the therapy is still open to debate. The study aimed to investigate the potential of methadone trough concentration (C_{trough})tobe a surrogate marker for such purpose. We conducted a 9-months prospective study to assess the relationship between C_{trough} and Dose (D) of methadone. A total of 115 subjects fulfilled the inclusion criteria and had given their consents to participate. Two (2) ml of the trough blood samples (C_{trough}) were taken and centrifuged within 4 hours from the time taken at 5,000 Gfor 5 minutes. The resulting serum samples were kept at -20°C until further analysis. The methadone concentration was determined by using a validated method for Methadone ELISA kit. The patients were subjected to another 2 follow ups at 3 months interval each and the same method of serum sampling was applied. Initial correlation reveals significant positive correlation between the two variables in every follow up, ranging between r=0.403-0.419 ($p<0.005$). Further regression analysis reveals that the coefficient of determination, r^2 was poor with only 15-17% of variation in the C_{trough} can be explained by the changes in clinical doses ($p<0.005$). Based on the results, we conclude that daily clinical dose poorly predicts methadone C_{trough} for the purpose of dosing adjustment and monitoring of therapy.

Keywords: Methadone dose, trough serum methadone concentration, methadone dose and serum concentration relationship.

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INTRODUCTION

The use of illicit drugs particularly the intravenous drug use (IDU) has been consistently shown a close relationship in communicating *Human Immunodeficiency Virus*(HIV)anditsfurther complicationsofacquired immunodeficiency syndrome (AIDS) in the population [1-4]. In many Asian countries including Malaysia, heroin is still regarded as the major used illicit opiates compared to other class of abused drugs which explains the 70% of HIV infected patients are amongst intravenous drug users [5]. It has also been estimated that 1 in every 5 intravenous (IV) drug users is HIV positive which make up approximately 20% of the IDUs [5,6]. The trend will nevertheless increase if no step taken to tackle the problem [7]. In view of this menace,

methadone maintenance therapy (MMT) was introduced in patients with opioid use disorders as one of the harm-reduction approaches [8-10]. Practicing MMT in various countries shows promising results [11-15]. The usage was initially introduced by Professor Vincent P. Dole and the late Professor Marie E. Nyswander back in 1965 [8]. In their now famous study, MMT was shown to prevent and relieve opioid craving while blocking its euphoric effects [9]. It normalizes the somatic, neurological as well as endocrinological dysfunctions associated with prolonged opioid use [10]. As results, studies have shown that commitment to such therapy reduced cravings and withdrawals which lead to the reduction in illicit drugs use and degrading notorious spread of HIVin the community [12, 14,16-18].

However determining an optimal dose is a major challenge [19,20] due to its complex disposition mediated by polymorphic enzymes, transporters and receptors [21-27]. The consequent pharmacokinetic variability makes similar doses not yielding similar plasma concentrations or clinical effects in different subjects. Its long half-life and wide inter-patient variability in its clearance also make methadone use difficult to optimize."Higher is better" notion generally holds through with methadone maintenance dose to ensure retention in programs. It was found that a dose of 50 mg/day was associated with higher retention rates compared to lower doses [28]. Similarly, patients maintained on 60mg/day or higher had better treatment outcomes and indeed, doses exceeding 100 mg/d have been used safely and effectively in long-term maintenance treatments [29]. Similar observations were also reported in other studies [30-32].

Nevertheless, several other studies failed to find a clear association between positive treatment outcomes and high doses. In Canada for instance, both higher and lower dosage protocols have been clinically implemented with parallel end results in different populations. Older and more motivated patients were given the low dose (40 mg) whereas higher doses (100 mg) were given to less motivated and more chronic users [33]. In an earlier studywhere a dose of 30 mg daily was used, it was reported that patients remained on treatment for 6 to 12 months and scored higher in terms of outcomes, such as reduced illicit heroin consumption, reduced arrest due to criminality and full-time employment compared to the dropouts [34] even at this low dose. A study reported on continuing use of illicit drugs and cravings despite high methadone dosage [35].

Blaney*et al* (1999) reported lack of significant difference in any of the outcome variables attributable to methadone doses [36]. Based on those findings, it has been suggested that the dosage of methadone should be individualized instead of relying solely on the population data.

In this study, we assessed dose-C_{trough} relationship in order to further propose on monitoring of methadone trough serum concentration (C_{trough}) instead of dose (D) solely, in patients with MMT. We also investigate other environmental factors which may contribute to the variability of both methadone clinical dose and its $C_{trough.}$ In conducting this study, we assumed that methadone C_{trough} at steady state reflects the receptor level of methadone concentration in exerting the clinical effects.

MATERIALS AND METHODS

Study Design

This is an observational prospective study. Patients were required to undergo twice follow-ups with 3 months period interval.

Ethics Approvals

The study was approved by the UniversitiSains Malaysia (USM) Ethical Committee and was registeredwith the National Medical Research Registry (NMRR-09-773-4587), Clinical Research Centre (CRC), Ministry of Health Malaysia. As the study was multi-centred, similar approvals were also obtained from the ethical committee at the International Islamic University Malaysia (IIUM).

Inclusion and exclusion criteria

The inclusion criteria include those aged more than 18 years old, exhibited high treatment compliance rate which should not be more than 20% of non-compliance rate, those have been under treatment for more than 6 months and willing to participate. The subjects were briefed on the study nature 7 objectives and signed the informed consent form. Those who were unfit and suffered from severe unstable psychiatry conditions were excluded from the study.

Clinical setting and protocol

This study was conducted prospectively for 9 months, starting from 1st of April 2010. . The study phase was divided into a baseline phase (BL), follow-up 1 (FU1) and follow up 2 (FU2). After signing of informed consents, the patients were interviewed using an adopted Brief Treatment Outcome Measure (BTOM) questionnaire [37]. This was used to gather socio-demographic, history of drug addiction, drug dependency patterns, other drug usage, other psychiatry related illness and other issues related to treatment. On their first visit, two ml blood was obtained just before their next dose (C_{trough}) to determine methadone concentration. Samples were left to coagulate at room temperature for 30-45 minutes (not more than 60 minutes). The tubes were taken for centrifugation at 5,000 g x 5 minutes and the supernatant or the serum was transferred to other pre-labelled empty tubes. These final samples were kept at -20°C until analysis. Other treatment observation during the first visit included respective dose and compliance rates, were assessed. Any significant comments from the physician were also recorded. Patients were then scheduled to the next two consecutive visits approximately three months apart.

For the next two visits, the same sampling methods and data collection protocols were employed. Patients were classified as dropouts if they failed to meet the original inclusion criteria, transferred to another methadone clinic by their own preference or assigned by the physician in-charged, hospitalized for more than a month, defaulted treatment for more than a month, jailed or died either due to medical problems or any other causes like motor vehicle accidents. Patients were considered as deviating if they were suddenly found non-compliant or they declined to participate during the process of data collection. Compliance rates were carefully assessed individually, especially for patients on take away doses.

Methadone C_{trough} determination.

The methadone $C_{trough}$was determined by using a validated methadone enzyme-linked immunosorbent assay (ELISA) kit [38]. All reagents and samples were brought into room temperature (18-26°C) for at least 30 minutes before use. This was to standardize the temperatures of the liquids involved. The reagents and calibrators will then be vortex-mixed for 15 seconds to produce a homogeneous mixture. The serum and the standard samples would also be re-centrifuged at 5,000 G for 1 minute before each use. Five micro litres of serum and standard samples were diluted at 1:100 in 1.5ml micro-centrifuge tubes with dilution buffer. These samples were again vortex-mixed for 30 seconds in order to produce a homogeneous mixture.

Ninety micro litres of each calibrator would be added into the first strip of plain micro-wells in duplicates. The same amount of serum and standard samples would be further added in duplicates based on the labelled grid which would have been prepared earlier. Thirty micro litres of the enzyme conjugate or methadone HRP would be systematically added into each well. The micro-plate would further be sealed to avoid evaporation. It would then be gently shaken on the shaker for about 1 minute with a rotary motion to produce an even mixture. 100 µl of the content would carefully be transferred into the methadone antibody coated wells according to the same grid by using a multiple channel pipette before being placed in incubation in the dark at room temperature for 1 hour.

Next would be the washing step where the content of the plate would be emptied and 300 µl of diluted wash buffer would be added into the wells. Another rotary shaking would be carried out for 10 seconds. The plate would then be inverted and vigorously slapped dry on the absorbent paper to remove any remaining liquid inside the wells. The washing steps would be repeated for another 2 times. This step is critical in ensuring that residual enzyme conjugates, would not skew results. After successful completion, 150 µl of TMB solution was added into each well and again incubated in the dark room temperature for another 30 minutes. Finally, 50 µl of stopping solution would be added into each well and shaken gently with rotary motion for another 5 seconds. Absorbance would be measured at the dual wavelengths of 450 nm and 650 nm. The reading would be done in no more than 30 minutes of yellow colouring development.

To interpret results, OD values of the NSB would be subtracted from each individual OD of the calibrators as well as the samples. To plot the calibration curve, the mean value of the zero calibrator (0ng/ml) would be calculated. The net OD value of the calibrators and samples (mean values of the duplicates) would then be divided by the mean OD value of the zero calibrator and multiplied by 100%.

$$\frac{[\text{O.D. calibrator (or sample)} - \text{O.D. NSB}]}{[\text{Mean O.D. zero standards} - \text{O.D. NSB}]} \times 100\%$$

$$= \% \text{ of maximal optical density} = B/B_o\ (\%)$$

The calibration curve would be plotted by using values of the percentage of maximal optical density which is calculated for the standards on the y-axis, and the methadone concentration (ng/ml) on the logarithmic x-axis. The methadone equivalent concentrations would be read from the calibration curve (Figure 1). Since the dilution method was applied earlier, the results of methadone equivalent concentrations would be multiplied by a factor of 100 to obtain the true serum methadone concentration.

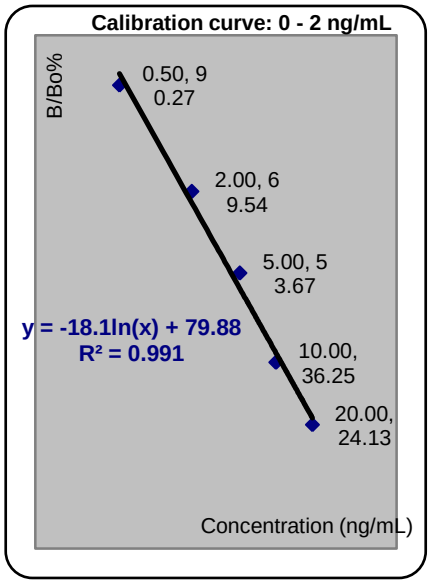

Figure 1: Sample of calibration curve plotted by using the calibrators

Statistical analysis

SPSS 18 was used as the statistical tool for data analysis. Mean of doses and C_{trough} was tabulated for each follow up. Pearson correlation analysis was applied to explore the initial relationship between $C_{trough}$and doses. Regression analysis was next employed to analyze the regression coefficient of the two measured variables.

RESULTS

Patients' characteristics

One hundred and twenty eight patients were screened however only 115 patients were enrolled and consented. In Follow Up 1 (FU1), 106 patients (92.0%) willingly complied with our study protocols and attended the third-month follow ups whereas another 27 patients defaulted in Follow Up 2 (FU2) increasing the drop-out rate to 31.0% from the baseline.We took approximately 4-5 weeks to complete the data collection at every phase and 3-6 patients were seen daily, for 5-7 days of a week.

Table 1: Demographic characteristics

No	Characteristics	Mean (SD)	n (%)
1	***Age (years old)***	**37.9 (8.1)**	
	18-29		10 (8.7)
	30-44		**82 (71.3)**
	45-59		23(20.0)
2	***Treatment duration (years)***	**2.2 (0.9)**	
3	***Gender***		
	Male		**114 (99.1)**
	Female		1(0.9)
4	***Source of income***		
	Full time		**75 (65.2)**
	Part time		26 (22.6)
	Pensioner		2 (1.7)
	No specific income		10 (8.7)
	Undetermined		2 (1.7)
5	***Monthly income (RM/month)***	**898.5 (572.1)**	
	500 and less		15 (13.3)
	501-1000		**57 (50.4)**
	More than 1000		20 (17.7)
	Undetermined		21(18.6)
6	***Marriage status***		
	Divorcee/separated		17 (14.9)
	Never married		**53 (46.5)**
	Married		44 (38.6)
7	***Living with***		
	Alone		20 (17.4)
	Spouse only		14 (12.2)
	Spouse and children		27 (23.5)
	Parents		**43 (37.4)**
	Siblings/ relatives / friends		11 (9.7)
9	***Housing type***		
	Rental house/room		**47 (40.9)**
	Own house		24 (20.9)
	Parents place		38 (33.0)
	Sponsored hostel		3 (2.6)
	No specific place / homeless		3 (2.6)
10	***HIVstatus***		
	HIV positive		13 (11.3)
	HIV negative		**102 (88.7)**
11	***Directly observed therapy (DOT)/ Take away patients (TA)***		
	DOT		50 (43.5)
	TA		65 (56.5)

Descriptive statistics of doses (D) and trough serum methadone concentrations (C_{trough}) are listed in Table 2. The minimum and maximum doses were the same across the 3 points of data collection, without drastic changes of the mean. These were held even with inclusion of dropouts (intention-to-treat). On the other hand, in terms of trough serum methadone concentrations, the minimum, maximum and the mean were slightly increased compared to the baseline. The average dose used was less than 70 mg/day and the mean of methadone C_{trough}measured was lessthan 400 ng/ml.

Table 2: Descriptive analysis of Clinical doses (D) and trough serum methadone concentrations, (C_{trough}) in each data collection point

Data collection point	n	Mean (SD)	
		Dose (mg/day)	C_{trough} (ng/ml)
BL	115	65.3 (26.2)	288.9 (175.3)
FU1	106	64.5 (25.4)	344.5 (190.5)
FU2	79	65.3 (27.5)	339.8 (198.4)

BL=Baseline, FU1=follow up 1, FU2=follow up 2, SD= standard deviation

Bivariate correlation analysis between clinical doses (D) and trough serum methadone concentration (C_{trough}).

Referring to Table 3, there was a consistent and significantpositive,moderate correlation **(r=0.403-0.419, p<0.001)** between clinical doses and their respective C_{trough}. We also performed regression analysis to further explore the relationship. Figures 2-4 show scatter plots of simple linear regression analysis for doses of methadone versus its serum concentration.

Table 3: Correlation analysis between clinical doses (D) and respective trough SMC (C_{trough})

N	Data collection points	Correlation, r [+]
115	Baseline	0.403**
106	Follow up 1	0.419**
79	Follow up 2	0.406**

***p value for correlation is <0.001*
[+] *Spearman's rho correlation analysis*

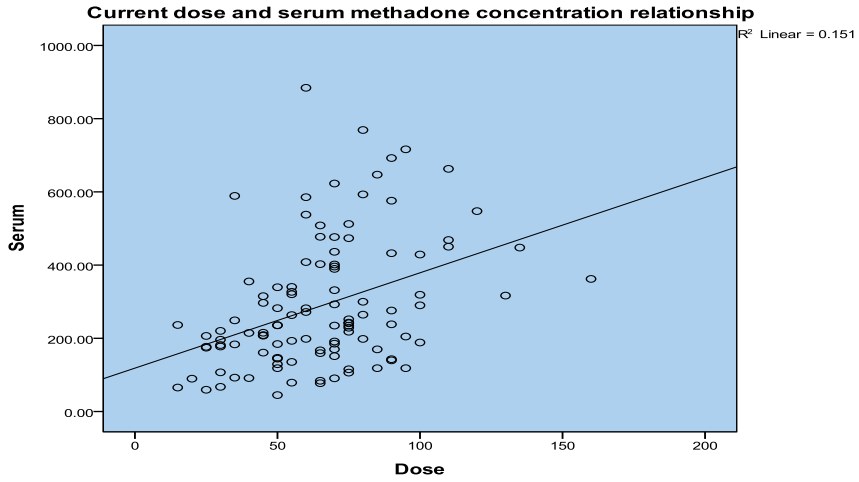

Figure 2: Regression analysis between clinical doses (D) with C_{trough} at baseline

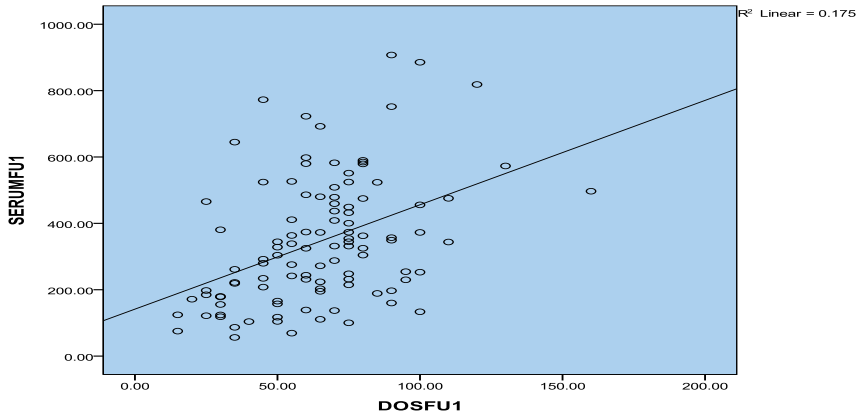

Figure 3: Regression analysis between clinical doses (D) with C_{trough}at Follow up 1

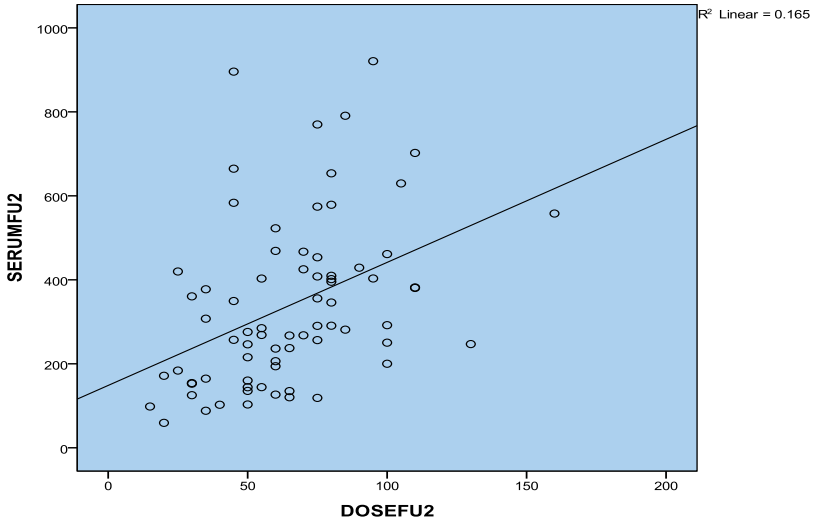

Figure 4: Regression analysis between clinical doses (D) with C_{trough} at Follow up 2

Even though there occurred a trend of increasing standard deviation (SD) with doses increased, it is evident that only 15.1-17.5% of trough serum methadone concentration was explained by the changes in clinical doses ($p<0.001$) (Table 4). The coefficient of determination, r^2 would not give an accurate description to determine serum concentration at given doses as clinical doses (D) only explain a little in terms of changes in serum methadone concentration (C_{trough}).

Table 4: Linear regression analysis between clinical doses (D) and trough SMC (C_{trough})

Data collection point	Coefficient of determination, r^2	Regression coefficient, B (95% CI)	*p value*
Baseline	0.151	2.60 (1.43- 3.78)	**<0.001**
Follow up 1	0.175	3.15 (1.82- 4.47)	**<0.001**
Follow up 2	0.165	2.93 (1.37- 4.49)	**<0.001**

Difference analysis in mean/median of trough SMC (C_{trough}) and methadone daily dose (D) in different categories of patients' demographic characteristic.

We further analyzed the patients according to their demographic characteristic (Table 5 and 6) in order to identify any confounding factors which may also contribute to the variability in trough C_{trough} and clinical doses (D). Patients who were positive with HIV AIDS reported use of significantly lower doses compared to negatively diagnosed patients ($p<0.05$). The mean C_{trough}in both categories however, did not reach the level of significance ($p<0.1$). Patients who admitted to involvement of other family members as opiate users had a higher mean C_{trough}compared to those who denied such an involvement ($p<0.05$). Similarly, a higher mean C_{trough}wasobservedamong patients on DOT compared to those on the Take Away (TA) regime ($p<0.05$). Patients who admitted to additional unknown doses of methadone have shown a significant lower mean of methadone dose compared to those without the extra doses of methadone but as expected, the difference in C_{trough} did not reach statistical difference. Other demographic characteristics did not seem to influence the mean of C_{trough} or doses. We further analyzed methadone C_{trough}-dose relationship after considering these probable co-factors (Table 7).

Table 5: Difference analysis in mean/median of trough SMC (C_{trough}) and methadone daily dose (D) in different categories of patients' demographic characteristic (categorical data)

Variables	Data Distribution[a]		n (%)	Mean/Median	Statistics	*p* value	n (%)	Mean/Median	Statistics	*p* value
	C_{trough}	D		C_{trough}(SD/IQR)				D (SD/IQR)		
Source of income										
Full time	0.000	0.032	75 (65.2)	219.12 (194.77)	2.107 (2)[c]	0.349	70 (60.9)	65.00 (25.00)	1.489 (2)[c]	0.475
Part time	0.200	0.200	26 (22.6)	289.53 (308.33)			26 (22.6)	72.50 (31.25)		
No specific income	0.200	0.200	12 (10.4)	242.72 (222.03)			12 (10.4)	55.00 (47.50)		
Living with HIVAIDS										
- HIV negative	0.001	0.200	95 (88.8)	263.94 (265.88)	-1.652[b]	0.098	102 (88.)	65.51 (23.47)	14.35 (0.51,28.20)[d]	**0.042***
- HIV positive	0.200	0.200	12 (11.2)	196.03 (74.97)			13 (11.3)	51.15 (25.34)		
Involvement of other family member										
- Not admit	0.200	0.200	54 (56.8)	284.65 (146.27)	-80.88 (-147.15, -14.60)[d]	**0.017***	57 (57.6)	63.33 (25.83)	-7.02 (-17.46,3.41)[d]	0.185
- Admit	0.200	0.200	41 (43.2)	365.53 (178.89)			42 (42.4)	70.36 (25.88)		
Current injecting behavior										
- 0-3 month	0.036	0.200	22 (20.0)	255.89 (170.51)	2.753 (3)[c]	0.431	22 (19.1)	55.00 (41.25)	2.671 (3)[c]	0.445
- 3-6 month	0.035	0.200	17 (15.5)	236.40 (311.18)			18 (15.7)	70.00 (32.50)		
- >6 month	0.001	0.010	52 (47.3)	211.96 (250.90)			50 (43.5)	67.50 (28.75)		
- > 1 year	0.200	0.200	19 (17.3)	290.05 (188.90)			20 (17.4)	55.00 (30.00)		
Directly observed therapy (DOT)/ Take away patients (TA)										
- DOT	0.200	0.200	39 (41.5)	357.14 (150.94)	74.91 (12.26, 137.56)[d]	**0.020***	42 (42.9)	69.05 (23.95)	5.30 (-5.32,15.73)[d]	0.329
- TA	0.200	0.050	55 (58.5)	282.22 (150.53)			56 (57.1)	63.84 (27.40)		
Methadone extra doses/not										
Without extra doses	0.001	0.017	102	239.68 (236.86)	-0.460[b]	0.645	107	70.00 (30.00)	-1.973[b]	**0.048***
With extra doses	0.000	0.200	8	228.08 (190.12)			8	47.50 (33.75)		

*C_{trough} = trough serum methadone concentration, D=Methadone daily dose, SD=Standard deviation, IQR=Inter quartile range **a**- Kolmogorov Sminorv test (p>0.05 indicate normally distributed data), **b**- Z stat for Mann Whitney test, **c**-χ^2 stat (df) for Kruskal- Wallis test **d**- Mean difference (95%CI) for Independent t-test, **e**-F value (df) for One way ANOVA test, *****-p value <0.05 shows significant difference.*

Table 6: Correlation matrix of trough SMC (C_{trough}) and methadone daily dose (D) at baseline in different patients' demographic characteristic (continuous data)

Variables	1	2	3	4	5	6	7	8	9	10	11	12
Treatment duration	1.000											
Years involved	0.031	1.000										
Age	0.085	**(0.853)**	1.000									
Income	0.044	0.095	0.087	1.000								
Age of first exposure	0.066	**(-0.347)**	0.070	0.083	1.000							
SDS scores	-0.019	**(-0.182)**	**(0.259)**	0.132	0.160	1.000						
HIV risk scores	-0.131	-0.056	0.039	-0.082	0.152	0.140	1.000					
Cigarrette smoking	-0.012	0.022	0.050	0.109	-0.173	0.040	0.026	1.000				
SFS scores	-0.097	0.160	0.062	0.180	**(-0.262)**	0.132	**(-0.201)**	-0.030	1.000			
PFS scores	-0.063	0.110	0.021	0.149	**(-0.245)**	**(0.203)**	**(-0.212)**	-0.145	**(0.385)**	1.000		
C_{trough}	0.016	-0.040	-0.093	-0.055	-0.144	-0.001	-0.081	0.110	0.078	0.041	1.000	
Dose	-0.036	0.048	0.025	-0.015	-0.128	-0.072	0.146	0.013	-0.029	0.014	**(0.403)**	1.00

*HIV- Human Immunideficiency virus, SFS –Social functioning Scores, PFS – Psychological functioning scores, **( )**- significant correlation with p value<0.05. All of the binary correlations were analyzed by using Spearman's rho correlation as all showed abnormally distributed data.*

Table 7: Dose-serum relationship after considering possible co-variates.

Selected cases	n	Spearman's rho correlation, r	Coefficient of determination, r^2	Regression coefficient, β (95% CI)	*p value*
HIV negative, without other family involvement, take away patients and without extra doses	37	**0.441***	0.195	2.64 (0.74 – 4.54)	**<0.001****

**Correlation is significant at the level of 0.05 (2-tailed)*

***Regression analysis is significant at the level of 0.001 (2-tailed)*

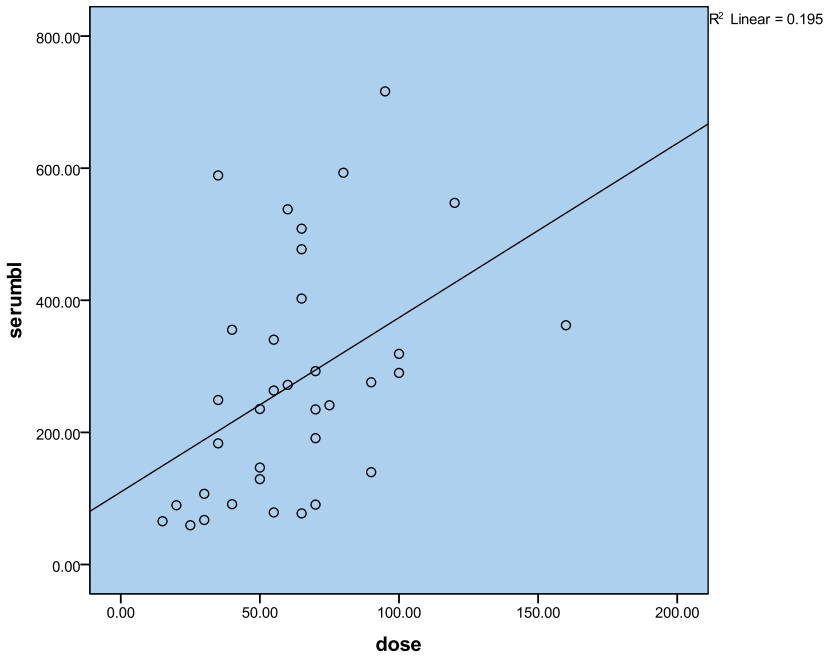

Figure 5: Serum-dose relationship after considering significant patients' characteristics

It was clearly shown (Figure 5) that approximately only 20% of the changes in serum methadone concentration can be explained by the changes in doses which are very much in support of prior analysis.

DISCUSSIONS

Methadone Maintenance Therapy

Several characteristics have especially made methadone suitable for maintenance therapy [39]. Methadone exhibits an excellent oral bioavailability that ranges between 80-90%. The drug also exhibits a long half-life allowing for a once daily dosing that can fit well into the patients' daily life. Patients will be able to lead a normal lifestyle and maintain productivity, without the deliberating craving. Another important criterion of methadone is its slow onset of action, which prevents the fluctuations of drug concentrations in the blood and brain, preventing withdrawal symptoms. This allows for a steady state "perfusion" of the drug at its site of action on the specific opioid receptors and other sites involved [40]. MMT is a life-long commitment. The longer the patients receive MMT the better the outcome and this has been used by detractors who saw MMT simply as replacing one dependency with another [41]. A longitudinal prospective cohort study showed a long term continuous improvement in the quality of life of patients on long durations that ranged from 3 to 12 months and this has also supported long term use of the treatment [42]. The benefits are maintained in patients with psychiatric co-morbidity who also showed improvements including longer treatment retention, reduction of illicit drug use and

reduction in HIV-related risks behaviour [43]. Similarly, providing methadone in incarcerated settings are also effective but the therapy has to be continued post-release [44]. It was introduced especially to prevent the wide spread of HIV- related risks such as needle sharing behaviour and illicit drug use. With low costs calculated per patient, MMT has been put as top priorities on reducing opioid dependencies in Malaysia.

Gossop M *et al* has undertaken a big study called the National Treatment Outcome Research Study (NTORS). The majority of patients in the study demonstrated improvements in each domain measuring illicit drug use, criminal behaviour, withdrawal symptoms, HIV AIDS risk behaviour, health status and overall socioeconomic position as well as quality of life [45]. A similar study conducted in Malaysia produced similar trends of success with improvements in the quality of life in patients undergoing MMT. Retention rate was 63.6% after 2 years and significant improvements were documented in terms of physical and psychological health, socio-economic status and existence of supportive environment ($p<0.001$) [46].

However, the dosing strategy of this therapy, in certain circumstances was open to doubt. With the hypothesis of a personalized methadone therapy and methadone C_{trough} may possibly be a surrogate marker for such purpose, we accordingly, conducted a 9-months prospective study to assess the relationship between C_{trough} and Dose (D) of methadone.

Relationship between current methadone clinical doses (D) with respective trough serum methadone concentration (C_{trough}).

Initial analysis yielded a significant fair correlation between clinical doses and methadone C_{trough} ($r=0.4$, $p<0.001$). However, on further regression analysis, only a poor relationship was observed between the two with only about 20% (r^2) of the changes in methadone serum concentration explained by changes in dose ($p<0.05$). This finding is not surprising given that methadone undergoes polymorphic metabolism mediated by several polymorphic enzymes like CYP3A4 and CYP2B6, apart from being influenced by the also polymorphic P-gp. Environmental factor could also play a role in this poor relationship. In our study, patients with HIV AIDS seemed to require lower methadone doses compared to HIV negative patients ($p<0.05$) to produce an essentially similar C_{trough} although this group of HIV positive patients received no anti retroviral therapy. A study suggested the development of methadone antibodies in HIV positive patients that increased serum methadone [47]. Further studies are however needed to validate the findings. DOT patients also yielded a higher concentration compared to those on take away regimes ($p<0.05$) probably because of a more ascertained administration of the methadone dose. A further difficulty occurred taking into consideration that eight patients admitted to extra doses of methadone. These patients had a significantly low methadone dose prescribed ($p<0.05$) for a comparable $C_{trough.}$ Mean doses at all the three follow up visits, were less than 70mg/day yielding a mean concentration of less than 400ng/ml. As alluded, this could be increased to clinical response especially taken into account our previous and other studies that suggested a minimum dose of 80mg/day and keeping serum racemic methadone concentration between 400-700ng/ml [48,49].

The monitoring serum concentrations of methadone has been previously suggested by Wolf *et al.* in (1992) who reported a high correlation between doses and plasma concentrations ($r=0.89$)

[50]. Subsequent studies however produced contradictory results. One study found a fair correlation (r=0.36) that improved with the exclusion of those with co-administered drugs (r=0.53). A poor correlation was however found by another group who studied patients with co-dependencies (r=0.25) [51]. Indeed, the correlation between methadone doses and methadone plasma/serum concentrations was variable (r=0.20-0.55, $p<0.05$) [49]. Another study that attempted to divide between patients with higher and lower doses found that patients on lower doses of methadone (<80mg) yielded a better correlation between dose and concentration (r=0.799, $p<0.001$) compared to patients on higher doses (r=0.004, p=0.980) [52]. The reports however failed to report regression analysis which would be useful to describe a bivariate relationship.

Pharmacogenomics of methadone

Genetic contribution [21, 49] are increasingly seen as important factor that can impact on the pharmacology of drugs including methadone. Most processes in pharmacology are not passive but mediated by some very specific proteins that in turn are products of gene expression. To exert its effect, drugs like methadone need to transverse membranes to reach their sites of action. Although most of these transfers are not simply passive diffusion across membranes/cells, generally, a lipid-soluble drug is favored. Methadone is a poorly water-soluble drug. Methadone, like any other exogenous substances, needs to be eliminated as it is not endogenous. For drug elimination, the kidneys play a pivotal role, excreting water-soluble substances which methadone is not. Prior to this renal elimination process, methadone undergoes metabolism mediated by several genetically polymorphic enzymes that include CY2B6 and CYP3A4. Prior to or subsequently, methadone is transported across cells and membranes with the help of some transporter proteins like Pgp. Methadone does not have a direct pharmacologic effect. To exert its effect, methadone needs to bind to some specific receptors that include the various forms of opiate receptors. All these mediators for transfers across cells and membranes, metabolism and effects are dependent on genes for their formation and functions and these genes are frequently polymorphic.

Methadone, is a drug with a large inter-individual variability and has a narrow therapeutic index. These are caused by the genetic polymorphisms in genes coding for transporter proteins (p-glycoprotein), methadone metabolizing enzymes and μ opioid receptors [21]. P-gp is a member of the subfamily B of the ATP-binding cassette (ABC) superfamily. It is a trans-membrane protein of 1280 amino acids that is composed of two homologous sequences, each containing six trans-membrane domains and an ATP binding domain . P-gp is encoded by the ABCB1 (MDR1) gene. Being a substrate, its effects are therefore influence by the genetic polymorphism of P-gp that may inhibit transmembrane transfers of the drug manifested by reduced plasma concentrations and effects. It has been postulated that gene ABCB1 polymorphisms may influence the plasma methadone concentration and dosing requirements [22], however, the findings were inconclusive [23]. As methadone is a lipid soluble drug, it therefore requires biotransformation before it gets eliminated. Several enzymes have been associated with its metabolism and they include CYP 3A4, CYP3A5, CYP2C9, CYP2C19, CYP 2D6, CYP 2B6 and CYP1A2. These enzymes are genetically polymorphic and their polymorphisms impact on methadone metabolism.

CYP3A4 isozyme, also found in the gastrointestinal tract, metabolizes methadone before it reaches systemic circulation causing a first pass effect on methadone. Genetic variations in CYP3A4gene influence the severity of side effects and methadone withdrawal. [24, 25]. Apart from CYP3A4, CYP2B6 isozyme that is found in the liver, with is also important in methadone metabolism. Although thought to be unimportant in drug metabolism, there is a growing interest towards CYP2B6 polymorphisms and its clinical significance. Its substrate list has expanded recently and there are now evidence for its cross-regulation with CYP3A4, UGT1A1 and several hepatic drug transporters by the nuclear receptors pregnane X receptor and constitutive androstane receptor [26].

Methadone acts on the μ opioid receptor (MOR) especially OPRM1[53]. MOR is also the main molecular target of the active biotransformation products of heroin (6-monoacetylmorphine and morphine), as well as most opiate and opioid analgesic medications such as oxycodone, hydromorphone, and fentanyl, each of which has major potentials for addiction [27]. Abuse of, and addiction to these MOR-directed agents constitutes a major addiction problem [54].

CONCLUSION AND RECOMMENDATION

Clinical dose was found to be poorly predicts trough serum methadone concentration which suggests that C_{trough} may not possibly be a surrogate marker to predict the clinical outcome of methadone. However, further studies are needed to substantiate the relationship between methadone C_{trough} and patients' genotypes in which we hope will chart future paths towards personalized medicine for methadone.

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