Investigation on μ-opioid receptor in Sera of Iraqi Male addiction Tramadol or Methamphetamine

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Abstract. In Iraq, Drug addiction especially on Methamphetamine, (Meth); common name Crystal, and Tramadol (Tra) has increased after the year 2003. It becomes a dangerous issue, due to their multi dangerous negative effects on the health, economic, social for human, finally, it causing death. The aim of the present study is too sought out and to investigate the μ-opioid receptor (MOR) in Sera of Iraqi Male Addiction Tra or Meth. To do this, the work enrolled on 180 heavy smokers Iraqi male individuals at aged range 15-43 years (from January 2018 to December 2018) they were classified to 3 groups: G1 who were healthy control; G2 who was addicted on Meth with a dose ranged (1 - 5.0 gm for duration 1 -5 years); G3 who were addicted on Tra with an average dose (2 - 5.0 g) for duration 1 -5 years. The addiction individuals were admitted to Ibn-Rashid Hospital in Baghdad city to get the treatment. The MOR Concentration was determined by ELISA Technique while the drug level in the serum was determined by High Performance Liquid Chromatography (HPLC). The results showed a highly significant decrease (p<0.0001) in the level of MOR of the two addicted groups in comparison with the healthy group, especially those who addicted to Meth more than the others who addicted to Tra. Also the results also showed a strong negative correlation between MOR and dose (r = -0.9022, -0.8989) and duration of addiction (r= -0.8989, -0.8809) the serum of G2, G3 from the above results, The biochemical factor MOR can be used as a good marker to identify and follow up the addicted person.

Key word: MOR, Methamphetamine, Tramadol, HPLC.

1. Introduction

Opioid receptors have seven-transmembrane G-protein-coupled receptors (GPCR) with opioids as ligands [1]. The endogenous opioids are dynorphins, enkephalins, endorphins, and nociception. Three opioid receptors the μ (MOR), δ (DOR), and κ (KOR) receptor subtypes were cloned in 1990 and the nociception or orpanining receptor (NOR), a fourth opioid receptor, was added to the list in 1994[2, 3]. Opioid receptors are distributed widely in the brain and are exist in the spinal cord, heart, liver, lungs and digestive system [4]. Various studies suggest that pharmacological interactions between opioid receptors play a decisive role in the interpretation of their physiological behaviors. There is a rich pharmacology history of opioids from extensive clinical experience and studies of mechanism with a
wide range of drugs, most of them MOR agonists such as meth, Tra acts by binding to μ-opioid receptors on neurons [5, 6]. It is also an inhibitor of serotonin-norepinephrine reuptake, all Tra enantiomers are MOR agonists, but its M1 metabolite, O-demethylate, is six times more active than Tra itself. All of these actions combine to cause analgesia [6]. Payne and Pasternak provided the effects of these agents that were not possible with animal models due to the subtle differences among various opioid drugs. Clinicians have understood, for example, that opioids do not function in every patient the same way [7]. Some patients have a good response to one drug while another patient may be better managed with another one. Side effects can also be seen with a particular drug, regardless of its analgesic activity. Also, Eddy’s study at the Lexington Kentucky Addiction Research Centre found that patients with a history of opioid abuse could distinguish between one opioid and the other [8]. These observations raised the question of how to reconcile them with the presence of a single receptor, especially as almost all drugs were binding on the receptor of MOR [9]. While the MOR is primarily aimed at opioid analgesics, the DOR and KOR also control pain and analgesia and the relative affinities of opioids for these receptors give these individual properties [10, 11]. The effects of opioids also relied on MOR, while DOR and KOR alter them through the arrangement of mood and stress reaction. Figure 1-6 showed that although MOR, KOR and DOR agonists are all analgesic, clinical studies and genetic models indicate that they are at different ends of mood continuums [12]. The agonists of MOR give rise to euphoria and stress. KOR agonists produce dysphoria at the other end of the hedonic continuum and are correlated with stress and depressive effect [13]. DOR is on the opposite end of the continuum describing mood and DOR agonists have antidepressant and anxiolytic activity [14]. Meth is N-methyl-1-phenylpropane-2-amine, is a type of highly synthetic drug addiction that fundamentally affects the neurotransmitters systems of the brain and results in feelings of alertness, energy, and euphoria. Tra is 2-[(Dimethyl amino) methyl]-1-(3-methoxyphenyl) cyclohexanol. Tra is an analgesic, mainly due to its metabolite, O-demethyl tramadol, which produces opioid effects. It is excreted mainly by the kidneys and the remainder by the focal route [15]. Once Meth or Tra opioid transfer through the bloodstream into the brain, the chemicals attack specific proteins on the surfaces of opioid-sensitive neurons μ-opioid receptors. Binding these opioids to the receptors result in the same biochemical brain processes that reward people with good feelings like eating and sex.

**Subjects, Material and Methods:**

This part of the study was performed in the period from January 2018 until December 2018. Two hundred and forty blood samples of male individuals at age 15-63 years were controlled in this study. One hundred eighty (180) Iraqi male individuals of them were selected for study and divided into (sixty ones addicted to Meth and the sixty others addicted to Tra. All of them were selected from Ibn-Rushed Hospital in Baghdad city. A detailed history was obtained from each one. sixty (60) non-addicted healthy subjects serve as control groups participate in this study and they judge to be healthy according to their history and physical examination. All individuals were heavy smokers Exclusion criteria were any disease e.g. diabetic, blood pressure, retinopathy, neuropathy, cardiopathy and any inflammation that was registered for any subjects under this study.

### 1.1. Collection of blood samples

Approximately 10 milliners of venous blood were drawn fasting from each subject, using 10 ml dispensable syringe. Samples were collected after fasting for 10-14 hours. The blood samples were transferred into a gel tube, left at room temperature for clothing, centrifuged at 3000 rpm for 10mm in order to collect serum. The serum was used for measuring cortisol level, lipid profile, drug (Meth or Tra). The serum was stored in Eppendorf tubes at - 20°C until time of use.
1.2. Methods: Determination of μ-Opioid Receptor (MOR) Level by ELISA:

principle:

The MOR level in serum was determined by using Antibodies Inc. kit which was applied on sandwich enzyme-linked immune-sorbent assay for the quantitative measurement of human MOR.

1.3. Calculation of results

The standard curve was generated by plotting the average absorbance at λ max 450nm Obtained for each of six standard concentrations (0, 0.625, 1.25, 2.5 5, 10 ng/ml) on the vertical (Y) axis versus the corresponding concentration on horizontal (X) axis. And a best-fit curve was drawn through the point on the graph.

Fig 1: Calibration curve of MOR.

1.4. Determination of total drug

The concentration of drug used by individuals under this study Meth (group 1; G1) and Tra (group 2; G2) was determined in their sera by using modified HPLC technique under the optimum condition which was established previously. Mobile phase was a mixture of Acetonitrile: Ammonium acetate ratio 20:80. The optimum conditions were column temperature 25°C pH =3.5, flow rate= 1mL.min-1. The wavelength was 270 nm by using the UV-detection. The HPLC instruments used was manufactured by Shimadzu, Kyoto, Japan equipped HPLC system which is used with RP-sunfire™ C18 (5 um ×4.6 x250 mm) column [16].

1.5. Statistical Analysis

Analysis of data was completed utilizing the available statistical package of SPSS-25 (statistical package for social science – version 25). Data were exhibited in simple measures mean and standard error (SE) and standard deviation (SD). The significance of differences of various means (quantitative data from different groups and control group) were tested utilizing analysis of variance (ANOVA). When p-value less than 0.001 this statistically considers a high significant. The correlation between two quantitative variables was finished with the scatter diagram of the distribution with the bivariate person's correlation item coefficient ® calculation.
2. Results and Discussion

The level of MOR was a very highly significant decreased in G2 group and G3 group (p=0.0001) in comparison with the control group (G1). Also, it shows the mean±SD for G2 was decreased highly significant in compare to G3 (p= 0.0001) which means the individuals addicted on Meth their MOR level were decreased less than those who addicted on Tra drug as shown in Table 1. In the other word Meth had a higher effect on MOR level and binding capacity of receptors than Tra drug. Because Tra drug is weaker than other opioids, the people tend to think they cannot develop an addiction. However, Tra is still one of an opioid and thus highly addictive [17]. Consequently, it is generally considered as a drug with a lower potential (acts as a mild opioid) for dependence than the other opioid drugs such as Meth and cocaine. In the last few years, new data has been reported and these data confirm that Tra dependence may occur when used daily for more than a few weeks/months [18, 19].

| Groups                  | [MOR] ng/ml |       |       |       |
|-------------------------|-------------|-------|-------|-------|
|                         | Mean±SD     | SE    | p-value |
| Control (G1) N=60       | 4.385±0.6305| 0.1151| 0.0001a |
| Addicted on Meth (G2) N=60 | 1.134±0.2001| 0.0365| 0.0001b |
| Addicted on Tra (G3) N=60 | 1.549±0.1706| 0.03114| 0.0001c |

The receptor concentration, MOR, in the serum was significantly lowered in the current study (p=0.0001). This conclusion is confirmed by the results in the Table 2, which shows a marked decrease in serum receptor concentration with increased duration of addictive. There was also a decrease in the concentration of the receptor significantly(p=0.012) with an increased dose of abuse in Table 3.

Table 2 : Mean±SD , p value and Conc.rang of MOR for two addictive groups on Meth and Tra addicts in different duration times of addiction .

| Duration of addiction (year) | [MOR] ng/ml |       |       |       |       |
|-----------------------------|-------------|-------|-------|-------|-------|
|                            | Addicted on Meth | Addicted on Tra |
|                            | Conc.rang | Mean±SD | p-value | Conc.rang | Mean±SD | p-value |
| 1.00±0.50                  | 1.408-1.3780 | 1.394±0.093 | 0.091 | 1.674-1.579 | 1.635±0.048 | 0.0001 |
| 2.00±0.50                  | 1.148-1.2950 | 1.228±0.071 | 0.0001 | 1.418-1.501 | 1.451±0.044 | 0.001 |
| 3.00±0.50                  | 0.988-1.34 | 1.122±0.118 | 0.0001 | 1.407-1.482 | 1.439±0.013 | 0.0001 |
| 4.00±0.50                  | 0.9488-1.227 | 1.033±0.098 | 0.0001 | 1.368-1.428 | 1.394±0.031 | 0.0001 |
| 5.00±0.50                  | 0.7971-0.911 | 0.871±0.052 | 0.0001 | 1.144-1.288 | 1.216±0.102 | 0.012 |
Table 3: Mean±SD, p value and Conc.rang of MOR for Meth and Tra addicts with different dose of opioid drugs.

| DOSE g  | MOR ng/ml | p-value | Conc. rang   | Mean±SD   | p-value | Conc. rang   | Mean±SD   |
|---------|-----------|---------|--------------|-----------|---------|--------------|-----------|
|         | Addicted on Meth |         |             | Addicted on Tra |         |
| 1.00±0.50 | 1.223-1.599 | 1.356±0.154 | 0.106 | -          | -          |
| 2.00±0.50 | 1.142-1.408 | 1.275±0.106 | 0.0001 | 1.601-1.815 | 1.6921±0.096 | 0.002 |
| 3.00±0.50 | 1.014-1.342 | 1.145±1.047 | 0.0001 | 1.501-1.768 | 1.6743±0.089 | 0.0001 |
| 4.00±0.50 | 0.949-1.005 | 0.981±0.021 | 0.0001 | 1.324-1.595 | 1.4287±0.066 | 0.0001 |
| 5.00±0.50 | 0.797-0.911 | 0.850±0.053 | 0.0001 | 1.07-1.2880 | 1.167±0.111 | 0.0001 |

High-dose or long-term use of opioids may also be causing additional mechanisms of tolerance becoming involved. This includes down-regulation of MOR gene expression, thus reducing the number of receptors present on the cell surface as opposed to the more short-term desensitization induced by β-arrestins (proteins that are important for regulating signal transduction in protein-coupled receptors with G) or G-protein signalling regulators, these proteins are multifunctional, GTPase-accelerating proteins that increase GTP hydrolysis, thus inactivating the protein and switching off the signalling pathways of G-protein-coupled receptors rapidly [20]. In addition, a long-term adaptation to the use of opioids drugs can be done by upregulated of glutamate and other brain pathways that the decreasing of the opioid drugs effects [21]. David. et al., found that the rats with repeated of amphetamine injection (for 7 days: short term) had increased MOR activity compared with control rats[22]. Also, they further agree with Shen .et al., have shown the increased the activity of MOR in rats subjected to repeated injection of Meth for 11 days (short term), this study indicated that the development of behavioural sensitization to Meth can be controlled by the MOR system [23]. The results also showed a strong negative correlation between MOR and dose and duration of addiction. Table 4,5 and Figure 2,3.

Table 4: Correlation between MOR with Dose (g) of opioid drugs and Duration of addiction in Meth & Tra addicts.

| Parameter                        | [MOR] ng/ml |
|----------------------------------|-------------|
|                                  | Addicted on Meth (G2) N=60 | Addicted on Tra (G3) N=60 |
|                                  | r  | p  | r  | p  |
| Dose (g) of opioid drugs Meth&Tra| -0.9022 | 0.0001 | -0.8492 | 0.0001 |
| Duration of addiction (year)     | -0.8989 | 0.0001 | -0.8809 | 0.0001 |

r = correlation coefficient
The results showed a strong negative correlation between MOR and dose and duration of addiction. The purity of the opioid drug itself will determine the dose, for example, Currently, the purity of Meth is considered to be high, between 60-90%. This means that the strength of the effect on the central nervous system (CNS) will be greater than the low dose. Abuse doses that are common are between 100-1000 mg a day. Chronic usage can be as high as 5000 mg a day. Meth's toxicity effect could be assessed by the effects of the drug on a specific target that could be organism, organ, tissue, or cell at the level of the entire organism; it could be quantified based on the lethal dose, 50% (LD$_{50}$) [24]. The LD$_{50}$ for Meth is 55 and 57 mg/kg in rats and mice, respectively [25,26]. While in animal models the LD$_{50}$ value for Tra is around 300-350 mg/kg body weight [27,28]. It is an important measure to estimation a degree of toxicity, but it may be fake because the toxic effects of Meth in humans vary from those in laboratory animals, they are modulated by environmental conditions, depending on age and behaviour, length of use, use of other substances and individual sensitivity [29].

**Fig 2:** The correlation between [MOR] and (1) Dose of opioid drugs ; (2) Duration of addiction In serum for Meth addicts.

**Fig 3:** The correlation between [MOR] and (1) Dose of opioid drugs ; (2) Duration of addiction In serum for Tra addicts.

In conclusion from the above results: The biochemical factor MOR can be used as a good marker to identify and follow up the addicted person. Furthermore, the modified HPLC technique with established
conditions is a simple, accurate, precision method to determine the concentration of Meth or Tra in the serum or in the urine of an addicted person.

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