THE POTENTIAL ROLE OF SERUM S100B PROTEIN AS A PREDICTOR OF ACUTE TRAMADOL OVERDOSE INDUCED SEIZURE

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ABSTRACT

Background: Tramadol poisoning is one of the major health problems worldwide especially in developing countries. Seizure is considered a serious and life-threatening manifestation of acute tramadol overdose. Recently, serum S100B protein has been shown to be a useful and specific neuro-biochemical marker of central nervous system damage. **Aim of the study:** To assess the role of serum S100B protein on admission as a predictor of seizure occurrence in patients with acute tramadol poisoning and to determine the factors affecting tramadol-induced seizure. **Participants and methods:** Thirty-three patients with acute tramadol poisoning, of both sexes, and 20 healthy sex- and age-matched volunteers as a control group were included in this study. The studied tramadol poisoned patients were classified into two groups; group I: Seizure group and group II: Non-seizure group. For all patients, demographic and poisoning data were recorded then clinical evaluation and investigations were performed. Serum S100B level was estimated on admission. **Results: :** There was a statistically significant difference between patients with seizures and patients without seizures regarding age group, gender, ingested tramadol dose, Glasgow coma scale (GCS), poisoning severity score (PSS) and administered naloxone dose. On the other hand, there was no significant difference between both groups in manner of poisoning, delay time, vital data, oxygen saturation, arterial blood gases & pH and hospitalization period. The mean serum S100B was significantly higher in tramadol poisoned patients when compared to the control group; moreover, it was significantly elevated in patients with seizures than patients without seizures. Serum S100B as a predictor of seizures showed AUC =0.863 (p=<0.001) at cut-off value >0.58 µg/L with 75 % sensitivity and 95.24 % specificity. **Conclusion:** Serum S100B could be used as a good predictor of tramadol-induced seizure and it could be useful to assess clinical severity in acute tramadol overdose. Moreover, young adult males, increased ingested tramadol dose, increased administered naloxone dose and decreased level of consciousness are considered risk factors of tramadol-induced seizures.

Keywords: Tramadol, seizures, poisoning, S100B, biomarker, predictor.

INTRODUCTION

Currently, opioids uses and their related morbidity and mortality are being one of the major health problems worldwide (**Eizadi-Mood et al., 2014**). Tramadol is atypical, synthetic and centrally-acting analgesic that is related to codeine and morphine in structure (**Duke et al., 2011 and Nasouhi et al., 2015**).

Tramadol was ranked as a second cause of acute poisoning after oxycodone in annual reports of the American Association of Poison Control Centers Toxic Exposure Surveillance (**Watson et al., 2003**). Furthermore, a study in Tehran, Iran

reported that tramadol-related deaths in 2008 were 32.5 times more than in 2005 **(Iravani et al., 2010).** In Egypt, the incidence of tramadol poisoning was increased in the last years. The Poison Control Center (PCC) of Ain-Shams University Hospitals, Cairo, Egypt recorded 1595 patients with acute tramadol poisoning in the period between October 2010 and September 2011 **(El-Masry & Tawfik, 2013 and Fouad et al., 2015).**

Tramadol produces its analgesic effects by dual mechanisms; opioid and non- opioid mechanisms **(Afshari & Ghooshkanehee, 2009)**. It has low affinity to μ-opioid receptors. Therefore, most of its analgesic effect is due to its non-opioid properties via reuptake inhibition of central monoamines especially serotonin and norepinephrine **(Taghaddosinejad et al., 2011 and Majidi & Nekouei-Fard, 2014).**

Acute tramadol poisoning manifestations include nausea, vomiting, constipation, lethargy, coma and respiratory depression. Tramadol overdose might also present with features of the serotonin syndrome due to serotonin-norepinephrine reuptake inhibition rather than its opioid effects; which may include neuromuscular hyperactivity (myoclonus and hyperreflexia), autonomic hyperactivity (tachycardia, hypertension and pyrexia) and altered mental state (agitation, excitement and later confusion) **(Pothiawala & Ponampalam, 2011; Boostani & Derakhshan, 2012 and Rahimi et al., 2014)**.

Seizure was reported in 15-35% of tramadol intoxicated cases; meanwhile, it could also occur in therapeutic doses. There are different mechanisms that could explain tramadol-induced seizure

as gamma-aminobutyric acid inhibitory pathways or histamine (H1) receptor involvement or reuptake inhibition of norepinephrine and serotonin. Although tramadol poisoning may be non-life-threatening but, if accompanied by seizures it has been associated with reports of increased morbidity and mortality **(Farzaneh et al., 2012 and Eizadi-Mood et al., 2014)**

Recently, some neuro-biomarkers as S100B, neurospecific enolase, Tau protein and glial fibrillary acidic protein have been investigated as diagnostic and prognostic markers of central nervous damage in acute poisoning **(Akdemir et al., 2014 and Sykes & Walker, 2016).** S100B is a calcium binding protein found primarily in astroglial cells and Schwann cells. It plays an important role in neurodevelopment, differentiation and brain construction. It increases following brain damage, which easily penetrates into cerebrospinal fluid then into the blood. A normal S100B level has constantly been applied to predict the absence of major CNS damage and increased serum S100B level is not related only to neuroglia damage but could also reveal the current failure of the blood brain barrier **(Liu et al., 2015 and Sykes & Walker, 2016).**

There is limited information about factors affecting tramadol-induced seizure and no available data evaluating the role of serum S100B in acute tramadol poisoning. Therefore, this study aimed to assess the role of serum S100B protein, on admission, as a predictor of seizure in patients with acute tramadol poisoning and to determine the factors affecting tramadol-induced seizures.

PARTICIPANTS & METHODS
Study design and ethical points:
This prospective study was conducted on thirty-three patients, of both sexes, with acute tramadol poisoning who were admitted to Tanta Toxicological Unit during the period from July 2014 to June 2015 and 20 healthy sex- and age-matched volunteers as a control group. The study was approved by Research Ethics committee of Tanta Faculty of Medicine. Written informed consents were obtained from adult conscious participants or guardians of teenagers after complete description of the study. All patients' data were recorded in special sheets and the privacy and confidentiality of these data and records were ascertained through coding system.

Patients:
All patients aged 18 years or more, of both sexes, with acute tramadol poisoning admitted within 24 hours, during the study period, were included. Exclusion criteria included: patients with previous or family history of epilepsy, traumatic head injuries, neuropsychiatric disorders and patients with history of other poisonings or drug co-ingestion as well as patients with positive urine screen test for substance abuse including; benzodiazepines, barbiturates, opiates, methadone, amphetamine and tricyclic antidepressants. Furthermore, transferred patients from other hospitals, patients with systemic co-morbidities including metabolic, endocrinal or cardiac causes and infections were also excluded from the study.

Electrocardiography (ECG), head computed tomography (CT) scan and laboratory investigations including liver and renal function tests, complete blood count (CBC), random blood glucose level, sodium (Na) and potassium (K) levels were performed for every participant to exclude the patients who did not fulfill the criteria of the study.

Diagnosis of tramadol poisoning was based on history, characteristic symptoms & signs and, urinary screening analysis of tramadol. The studied tramadol poisoned patients were classified into two groups; Group I (Seizure group; patients having seizures) and Group II (Non-seizure group; patients do not having seizures). Diagnosis of seizure was based on jerky movements of the whole body, tonic/clonic spasms and convulsions (**Eizadi-Mood et al., 2014**). All volunteers of the control group gave negative history for taking any drugs and had negative urinary immunoassay for substances of abuse.

Methods:
For all patients, demographic data (age, sex, time of admission), poisoning data (name of drug, dose, route of exposure, mode of poisoning, delay time between exposure and arrival to hospital "pre-hospitalization period") and onset of seizures were recorded.

Clinical evaluation was performed for all patients including initial vital signs (blood pressure, pulse rate, respiratory rate and temperature), initial Glasgow Coma Scale (GCS), and poisoning severity score (PSS). Additionally, the administered naloxone dose, endotracheal intubation need and hospitalization period were documented. Serum S100B level, arterial blood gases (ABG) and pH were measured for all patients on admission and before treatment.

Three milliliters venous blood samples for S100B estimation were collected immediately on admission in plain test tubes. The whole blood was allowed to clot at room temperature for 30 min. Then, the samples were centrifuged at 3000 rpm for 10 min. Following centrifugation, the serum was separated and deeply stored at -80 °C until analysis for maximum of 6 months.

Quantification of human serum S100B protein was performed in accordance with the direction of manufacture by double antibody sandwich enzyme linked immunosorbent assay (ELISA) using Human S100B ELISA kit (Shanghi Sunred Biological Technology Co., Ltd, Shanghai, China). The results were expressed as µg/L. Also, one-milliliter arterial blood sample was taken on heparinized tube for estimation of arterial blood gases and pH.

Moreover, 20 ml of urine samples were collected from each patient on admission in clean dry sterile disposable containers, labelled with patient's name, serial number and date of taking the sample then were preserved at -20°C**.**

Statistical analysis

The collected data were organized and statistically analyzed using SPSS software computer package for windows version 22 (SPSS Inc., Chicago, Illinois, USA). For quantitative data, the Shapiro-Wilk test for normality was performed. For data that were not normally distributed median and interquartile range (expressed as 25th - 75th percentiles) were calculated and Mann-Whitney test was used for comparison between groups and Spearman's correlation was performed. For normally distributed data, values were expressed as mean ± standard deviation and independent sample student t- test was used for comparison. One sample t- test was used to compare S100B in the studied patients to the control group. For qualitative data, Pearson's Chi-square or Fisher's Exact tests were used as appropriate **(Dawson-Saunders & Trapp, 2001).** The receiver operating characteristic (ROC) curve was used to calculate cut-off value, sensitivity and specificity of S100B level according to **Delong et al. (1988).** Significance was adopted at $p < 0.05$ for interpretation of results of tests.

RESULTS

Thirty-three patients with acute tramadol poisoning were included in this study. The ages of the studied patients ranged from 18 to 46 years with a mean age 25.6 ± 9.3 years and most of the studied patients were young adult males aged between 21 to 40 years (63.6%). Toxicological data revealed that, the route of exposure was oral in all cases. The majority of cases (63.6%) were intoxicated due to suicidal attempts followed by tramadol abusers (27.3%). The mean delay time was 5.3 ± 2.1 hours. The ingested tramadol dose in the studied patients ranged from 150 to 3000 mg with a

median of 800 mg. The hospitalization period ranged from 24 to 125 hours and its median was 72 hours (**table 1**).

There was statistically significant difference between patients with seizures and patients without seizures regarding age decades, gender and ingested tramadol dose. However, there was no significant difference between both groups regarding the manner of poisoning, delay time or hospitalization period (**table 1**).

Table (1): Demographic and toxicological findings of the studied patients (n=33)

Variables		Group I Seizure group (n=12)	Group II Non-seizure group (n=21)	Total studied patients (n=33)	Test statistic	p value
Age (years)	Range	25.0-31.0	18.0-46.0	18.0-46.0	t= -1.484	0.151
	Mean ± SD	28.0±2.7	24.1±11.4	25.6±9.3		
Age decades N (%)	≤20Years	0 (0.0%)	9 (42.9%)	9 (27.3%)	X^2_{FE}= 10.600	0.003*
	21-40 Years	12 (100.0%)	9 (42.9%)	21 (63.6%)		
	>40-60 Years	0 (0.0%)	3 (14.3%)	3 (9.1%)		
Gender N (%)	Male	12 (100.0%)	9 (42.9%)	21 (63.6%)	X^2_{ChS}= 8.448	0.004*
	Female	0 (0.0%)	12 (57.1%)	12 (36.4%)		
Manner of poisoning N (%)	Accidental	0 (0.0%)	3 (14.3%)	3(9.1%)	X^2_{FE}= 1.738	0.487
	Suicidal	9 (75.0%)	12 (57.1%)	21 (63.6%)		
	Addict	3 (25.0%)	6 (28.6%)	9 (27.3%)		
Tramadol dose (mg)	Range	200 - 3000	150 - 1600	150-3000	Z_{MW}= 2.012	0.044*
	Median	1200	600	800		
	IQR	650 - 2125	400 - 900	400-1200		
Delay time (hours)	Range	3.0-10.0	2.5-7.0	2.5- 10.0	t= -2.015	0.053
	Mean ± SD	6.3 ± 2.6	4.8 ± 1.6	5.3 ± 2.1		
Hospitalization period (hours)	Range	24.0 - 120.0	24.0 - 125.0	24.0 - 125.0	Z_{MW}= -0.637	0.542
	Median	72.0	55.50	72.0		
	IQR	38.0-98.0	25.5-106.5	30.0-98.0		

SD=Standard deviation, t=Independent samples T test, X^2_{FE} = Fisher's Exact test, X^2_{ChS}= Pearson's Chi-square test for association, IQR=Interquartile range, Z_{MW}=Mann-Whitney test, *Significance at p<0.05.

Tables (**2 and 3**) demonstrated that pulse rate, temperature and blood pressure were within normal ranges in most of all studied cases (63.6%, 81.8% and 90.9% respectively), while tachycardia was recorded in 27.3% of cases. Bradypnea and respiratory acidosis occurred in 54.5% of cases per each. Also, hypoxia was recorded in 63.7% of studied patients. Seizure was recorded in 12 patients (36.36%). The seizures in all patients were generalized tonic-clonic and developed within 24-hours post tramadol ingestion. Patients with seizures had significantly lower GCS and severe PSS than patients without seizures. Meanwhile, no statistical significant difference was observed between the two studied groups as regards vital data (blood pressure, pulse rate, respiratory rate and temperature), oxygen saturation, arterial blood gases and pH.

Table (2): Clinical findings of the studied patients (n=33) on admission

Variables		Group I Seizure group (n=12)	Group II Non-seizure group (n=21)	Total studied patients (n=33)	Test statistic	P value
GCS (3-15)	Range	3-8	3-11	3-11	Z_{MW} = -3.948	< 0.001*
	Median	3	9	8		
	IQR	3.0-4.0	8.0-10.0	3.0-9.0		
PSS N (%)	Moderate	0 (0%)	12 (57.1%)	12 (36.4%)	X^2_{ChS} = 8.448	< 0.002*
	Severe	12 (100%)	9 (42.9%)	21(63.6%)		
Pulse Rate (beats/min) N (%)	Normal	9 (75.0%)	12 (57.1%)	21 (63.6%)	X^2_{FE}= 1.738	0.487
	Bradycardia	0 (0.0%)	3 (14.3%)	3 (9.1%)		
	Tachycardia	3 (25.0%)	6 (28.6%)	9 (27.3%)		
Blood Pressure (mmHg) N (%)	Normal	9 (75.0%)	21 (100.0%)	30 (90.9%)	X^2_{ChS} = 3.146	0.076
	Hypotensive	3 (25.0%)	0 (0.0%)	3 (9.1%)		
Respiratory Rate (breaths/min) N (%)	Normal	6 (50.0%)	9 (42.9%)	15 (45.5%)	X^2_{ChS} = 0.157	0.692
	Bradypnea	6 (50.0%)	12 (57.1%)	18 (54.5%)		
Temperature (ºC oral) N (%)	Normal	9 (75.0%)	18 (85.7%)	27 (81.8%)	X^2_{ChS} = 0.089	0.765
	Fever	3 (25.0%)	3 (14.3%)	6 (18.2%)		

GCS= Glasgow coma scale, PSS= Poisoning severity score, IQR= interquartile range, Z_{MW} = Mann-Whitney test, X^2_{ChS}= Pearson's Chi-square test for association, X^2_{FE}= Fisher's Exact test, * significant at $p < 0.05$

Moreover, the median of administered naloxone dose in the studied patients was 2.4 mg (range 0.4 - 10.8 mg). The administered naloxone doses were significantly higher in patients with seizures than in patients without seizures with median of 6.8 vs 2.4 mg respectively (p < 0.017). Only three patients (9.1%) required endotracheal intubation with no significant difference between the two studied groups **(table 3).**

As shown in **table 3**, the mean serum S100B of the studied patients was 0.50 ± 0.13 (0.30 - 0.81 μg/L). It was significantly higher than that of the control group 0.08 ± 0.02 (0.02 - 0.1 μg/L). Also, the mean serum S100B was significantly higher in patients with seizures than in patients without seizures (p <0.001).

Table (3): Laboratory findings on admission, endotracheal intubation and administered naloxone dose of the studied patients (n=33)

Variables		Group I Seizure group (n=12)	Group II Non-seizure group (n=21)	Total studied patients (n=33)	Test statistic	P-value
Oxygen saturation N (%)	Normal (≥ 95%)	3 (25.0%)	9 (42.9%)	12 (36.4%)	$X2_{FE}=$ 4.519	0.090
	Mild hypoxia (90-94%)	3 (25.0%)	9 (42.9%)	12 (36.4%)		
	Severe hypoxia (< 75%)	6 (50.0%)	3 (14.3%)	9 (27.3%)		
pH	Range	6.90 - 7.32	6.85 - 7.35	6.85 - 7.35	$Z_{MW}=$ -1.014	0.310
	Median	7.09	7.23	7.17		
	IQR	6.95 - 7.25	7.03-7.28	7.00-7.28		
Acid-base status N (%)	Normal	0 (0.0%)	3 (14.3%)	3 (9.1%)	$X2_{FE}=$ 4.018	0.252
	Metabolic acidosis	3 (25.0%)	6 (28.6%)	9 (27.3%)		
	Respiratory acidosis	9 (75.0%)	9 (42.9%)	18 (54.5%)		
	Respiratory & metabolic acidosis	0 (0.0%)	3 (14.3%)	3 (9.1%)		
Serum S100B level (µg/L)	Range	0.38 - 0.81	0.30 - 0.60	0.30 - 0.81	t= - 4.593	< 0.001*
	Mean ± SD	0.61 ± 0.13	0.44 ± 0.08	0.50 ± 0.13		
Endotracheal Intubation N (%)	No	9 (75.0%)	21(100.0%)	30 (90.9%)	$X2_{ChS}=$ 3.146	0.076
	Yes	3 (25.0%)	0 (0%)	3 (9.1%)		
Administered Naloxone dose (mg)	Range	0.4 – 10.8	1.2 - 4.4	0.4 - 10.8	$Z_{MW}=$ 2.377	0.017*
	Median	6.8	2.4	2.4		
	IQR	2.8 - 10	1.2 – 4.0	1.2 - 4.8		

IQR: interquartile range, Z_{MW} = Mann-Whitney test, X^2_{ChS} = Pearson's Chi-square test for association, X^2_{FE} = Fisher's Exact test, * Significant at p< 0.05.

Likewise, serum S100B showed significant positive correlations with delay time, PSS, administered naloxone dose and a significant negative correlation with GCS. However, no significant correlations could be detected between serum S100B and ingested tramadol dose, intubated cases or hospitalization period **(table 4).**

Table (4): Spearman's correlation between serum S100B with some relevant variables

Variables		Serum S100B (µg/L)
Delay time (hours)	r	0.551
	P	0.001*
GCS	r	- 0.727
	P	<0.001*
PSS	r	0.636
	P	<0.001*
Ingested tramadol dose (mg)	r	0.319
	P	0.070
Hospitalization period (hours)	r	0.277
	P	0.396
Administered naloxone dose (mg)	r	0.439
	P	0.011*
Intubated cases	r	0.342
	P	0.051

GCS: Glascow coma scale, PSS: poisoning severity score
r: correlation coefficient of Spearman's correlation, * significant at p<0.05.

Table (5) and Fig (1) showed the results of analysis of receiver operating characteristics (ROC) curve of serum S100B for predicting seizure occurrence in acute tramadol poisoning. The serum S100B level had 75.00% sensitivity and 95.24% specificity at cut-off value >0.58 µg/L and showed AUC =0.863, with a 95% confidence interval (p <0.001).

Table (5): Best cutoff, sensitivity, specificity, and area under the curve (AUC) values of serum S100B level for predicting the occurrence of seizure (95% confidence interval (CI))

Area under the ROC curve (95% CI)	0.863 (0.699 to 0.957)
z statistic	4.508
P value	<0.001*
Best cut off value (µg/L)	> 0.58
Sensitivity	75.00 %
Specificity	95.24 %
Positive predictive value	90.00%
Negative predictive value	86.96%

* Significant at p < 0.05
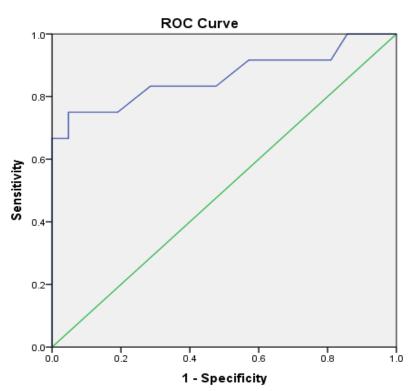
Figure (1): ROC curve analysis of serum S100B level as a predictor of occurrence of seizure. Area under the curve = 0.863, P <0.001*, sensitivity 75.00 % & specificity 95.24 % at cut off value >0.58 µg/L.

DISCUSSION

Tramadol is a frequently used opioid for management of moderate to severe pain (**Pothiawala & Ponampalam, 2011).** Although, tramadol is considered a safe analgesic but recently, reports of tramadol abuse, dependence, poisoning and deaths have been increasing, as it is still the only clinically accessible opioid in most countries (**Brinker et al., 2002; Tjaderborn et al., 2007 and Elkalioubie et al., 2011**).

Seizures are one of the most serious manifestations of tramadol therapeutic use, abuse or overdose. Seizures may induce complications such as hypoxia, trauma, muscular damage, rhabdomyolysis, renal failure and even death (**Shadnia et al., 2012; Eizadi-Mood et al., 2014 and Rahimi et al., 2014**). This study showed that seizures were significantly reported in

young adult males aged from 21 - 40 years, which is in line with the studies of **Talaie et al. (2009), Petramfar & Haghighi, (2010) and Ahmadi et al. (2012).** This could be attributed to, the more consumption of tramadol by male subjects to improve the premature ejaculatory function and extend the sexual orgasm (**Fawzi, 2010**). However, other studies did not demonstrate any significant difference in seizure incidence as regards age and gender (**Taghaddosinejad et al., 2011 and Rahimi et al., 2014**).

The current study demonstrated that the ingested tramadol dose in seizure group ranged from 200 to 3000 mg with median of 1200 mg, which was significantly higher than cases without seizures. This result partially agreed with **Taghaddosinejad et al. (2011)** who reported that the mean ingested tramadol dose accompanied

with seizures was 1511 ± 135.3 (range 200–7000 mg). Meanwhile, **Rahimi et al. (2014),** who studied patients with acute tramadol poisoning in Tehran, found significant decrease of ingested dose in cases with seizures than those without seizures (1395.7 ± 218.3 vs. 2500.7 ± 390 mg respectively). This discrepancy between the obtained results and those of Rahimi et al. could be attributed to genetic polymorphism. On the other hand, **Talaie et al. (2009)** demonstrated that there was no significant difference between patients with and without seizures in ingested tramadol dose and they attributed that to alcohol co-ingestion in cases with seizures.

Oral route was the only route of intoxication in this study and this is in accordance with the results of other studies (**Shadnia et al., 2012; Hassanian-Moghaddam et al., 2013 and Hassanian-Moghaddam et al., 2015**) which showed that oral route is the most common route of tramadol administration. This can be explained by the availability of tramadol oral forms as reported by **Fouad et al. (2015**).

The present study revealed that, the mean delay time was longer in seizure group than in non-seizure group (6.3 ± 2.6 vs 4.8 ± 1.6 hours respectively). This partially agreed with **Rahimi et al. (2014)** who demonstrated significant increase in delay time in seizure cases than cases without seizure (330.3 ± 53.5 vs. 257.3 ± 30.1 min respectively).

Additionally, intentional poisoning was the most common mode of poisoning followed by drug abuse in our study. This finding is in agreement with **Shadnia et al. (2008), Taghaddosinejad et al. (2011) and**

Rahimi et al. (2014). The median of the hospitalization period was 72 hours (range: 24 - 125 hours) which was in line with **Ghasempouri et al. (2014)** who found that the mean of hospitalization period was 1.24 day (range: 1 - 5 days). There was no significant difference between both groups as regards manner of poisoning and hospitalization period, which agreed with the results of **Rahimi et al. (2014).**

The majority of patients in the present study had signs of respiratory depression as bradypnea, respiratory acidosis in 54.5% per each and hypoxia in 63.7% of the studied patients. This finding was partially in accordance with **Hassanian-Moghaddam et al. (2013) and Rahimi et al. (2014)** who demonstrated respiratory problems as dyspnea and decreased partial pressure of oxygen in all tramadol-poisoned patients due to tramadol induced respiratory depression. Tachycardia was recorded in 27.3% of patients in this study that could be attributed to noradrenergic reuptake inhibitory effects of tramadol. This result coincided with **Ryan & Isbister (2015)** who found tachycardia in 38% of patients. However, no significant difference could be detected between both groups regarding vital data, oxygen saturation, arterial blood gases and pH, which coincide with the results of **Rahimi et al. (2014).**

Seizures were recorded in 36.36% of cases in the current study. This was comparable with previous studies which reported that seizures develop in approximately one-third of cases with tramadol overdose (**Shadnia et al., 2008 and Taghaddosinejad et al., 2011).** Despite, **Ryan & Isbister (2015)** recorded lower frequency of

seizures in tramadol poisoned patients (11%), but, other studies by **Jovanovic-Cupic et al. (2006), Talaie et al. (2009)** and **Rahimi et al. (2014)** recorded higher seizure frequency (54.4%, 46.2%, and 47.9% respectively) in acute tramadol poisoned patients.

This variation of seizure incidence could be attributed to many factors such as the different affinity of tramadol two enantiomers (+) & (−) for the opioid receptors and monoamines reuptake (**Potschka et al., 2000 and Beyaz et al., 2016)**. Additionally, tramadol is metabolized by cytochrome P450 (mainly CYP2D6 isomer) to its active metabolite (O-desmethyltramadol) which has serotonergic activity and can induce seizures. The impact of CYP2D6 polymorphism plays an important role in the different clinical presentations of tramadol toxicity (**Becquemont, 2009 and Boostani & Derakhshan, 2012).**

This explanation could be supported by the results of **Ibrahim et al. (2015)** who found a significant relation between tramadol's metabolite level and clinical severity among tramadol intoxicated Egyptian patients as 60% of the studied patients were extensive metabolizers, 23% were ultrarapid and 17% of cases were poor metabolizers.

Patients with seizures had significantly low GCS with median of 3 (range 3 − 8) and severe PSS than patients without seizures. Likewise, **Fouad et al. (2015)** reported that 85.7% of intensive care cases and 44.4% of non-survivor cases of tramadol poisoned patients had seizures and their GCS were between 3 and 8.76. Hence, this extreme drowsiness and coma may occur following seizures

(**Shadnia et al., 2008).**

In the present study, the administered naloxone dose was significantly higher in seizure group than in non-seizure group. The seizureogenic effect of naloxone in tramadol overdose was controversy. Some studies demonstrated that naloxone markedly increased the incidence of seizures (**Van de Kamp, 1986 and Taghaddosinejad et al., 2011).** Furthermore, **Farzaneh et al. (2012)** reported that naloxone could increase the seizurogenic effect of tramadol in high-risk patients**.** On the other hand, some studies found that naloxone might reduce tramadol induced seizure effects (**Yang et al., 2010 and Eizadi-Mood et al., 2014**). Although, the neuroprotective effect of naloxone was poorly understood, the antagonistic effect on opioid receptors could reduce cytokines production and glial activation (**Liu & Hong, 2003).** However, **Omrani et al. (2007)** revealed that naloxone did not alter the seizurogenic effects of tramadol.

To the best of the authors' knowledge, the present study was the first study to assess the role of S100B protein as a predictor of tramadol induced seizure. The current study demonstrated that, patients with acute tramadol overdose with and without seizures had significantly higher serum S100B levels when compared to the control group. Similarly, **Ambrozic et al. (2008**) found that serum S100B was significantly higher in acute benzodiazepine patients when compared to control group.

Moreover, patients with seizures had significantly higher serum S100B than those without seizures. Many previous studies have documented elevation of serum S100B in patients

with seizures and epilepsy (**Kacinski et al., 2012 and Dogan et al., 2013**). This could be explained by astrocytic reaction aiming to promote neuronal stability and protection (**Oses et al., 2004**). Elevated S100B serum levels may be an early event in seizure development because of blood brain barrier (BBB) leakage (**Marchi et al., 2007**).

Furthermore, serum S100B might be released to peripheral circulation as a result of hypoxic brain damage. Since, serum S100B is found in high concentration of astroglial cells, which are very sensitive to hypoxic stress. Therefore, serum S100B may indirectly reflect neuronal damage (**Bottiger et al., 2001**). This could be supported by significant increase of serum S100B in patients who had low Glascow coma scale (GCS) and severe poisoning severity score (PSS) on admission. Additionally, the majority of patients in the present study had signs of respiratory depression and hypoxia. Previous studies have reported the diagnostic and prognostic value of serum S100B in brain hypoxia due to carbon monoxide poisoning, benzodiazepine overdose, organophosphate poisoning and aluminum neurotoxicity (**Ambrozic et al., 2008; Yardan et al., 2009 and Cakir et al., 2010**). The role of tissue hypoperfusion, in the elevation of serum S100B, in the current study was unlikely as the majority of cases (90.9%) had normal blood pressure.

Moreover, ROC curve analysis showed that high serum S100B might be useful in prediction of tramadol-induced seizure. Above the cutoff value of 0.58 µg/L, serum S100B had 75 % sensitivity and 95.24 % specificity. This means that above level of 0.58 µg/L, S100B could predict seizures in 75% of cases and below these level, seizures could be excluded in 95.24 % of cases.

CONCLUSIONS:

Although tramadol seems to be a safe analgesic, it might cause subtle brain injury. Serum S100B is a good predictor of tramadol-induced seizure and it could be used as a useful biomarker to assess clinical severity in acute tramadol overdose. Additionally, young adult males increased both of ingested tramadol doses and administered naloxone dose, decreased level of consciousness and severe tramadol poisoning were considered risk factors of tramadol-induced seizure.

LIMITATION OF THE STUDY& RECOMMENDATIONS

One of the limitations in this study was our inability to follow up the patients which prevented us from evaluation of the prognostic value of serum S100B in delayed neurological sequelae after patient's discharge. Other limitations are the small sample size and absent tramadol blood level which prevented us from clarifying its role in seizure incidence. Therefore, we suggested performing a larger prospective study for acute tramadol poisoned patients taking into consideration a good follow up to evaluate the role of serum S100B as a diagnostic and prognostic biomarker. Also, we suggested serial measurements of serum S100B to be performed to verify the best time of sampling in acute tramadol poisoning.

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<u>الملخص العربى</u>

الدور المحتمل لمستوى بروتين ال S100B
بمصل الدم للتنبؤ بحدوث التشنجات فى حالات التسمم الحاد بعقار الترامادول

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خلفية علمية: يعد التسمم بعقار الترامادول من أهم المشكلات الصحية التى تواجه العالم بشكل عام و الدول النامية بشكل خاص. وتعتبر التشنجات التى قد تصاحب التسمم الحاد بعقار الترامادول من أخطر الأعراض التى قد تهدد حياة المريض. وقد تبين مؤخرا أن مستوى بروتين الS100B بمصل الدم يمكن أن يستخدم كأحد المؤشرات العصبية الكيميائية و الحيوية لتحديد مدى تضرر (إصابه) الجهاز العصبى المركزى.

الهدف من البحث: تقييم دور بروتين S100B عند دخول المريض كمؤشرلاحتماليه حدوث التشنجات المصاحبة للتسمم بعقار الترامادول. و كذلك تحديد العوامل المؤثرة على حدوث التشنجات المصاحبة للتسمم بعقار الترامادول.

طريقة البحث: تمت هذه الدراسة على ثلاثة و ثلاثين مريضا مصابين بالتسمم الحاد بعقار الترامادول و عشرين من المتطوعين الأصحاء كمجموعة ضابطه وقد تم تقسيم المرضى المصابين بالتسمم الحاد بعقار الترامادول إلى مجموعتين: المجموعة الأولى تضم المرضى المصابين بتشنجات و المجموعة الثانية تضم المرضى الغير مصابين بتشنجات. وقد تم تسجيل بيانات المرضى الديموغرافية وبيانات خاصة بالتسمم كما تم فحص المرضى و عمل التحاليل اللازمة عند الدخول وأيضاً تم قياس مستوى بروتين S100B بالدم.

النتائج: أظهرت نتائج الدراسة وجود فروق ذات دلالات إحصائية بين المجموعتين قيد الدراسة في كل من الفئة العمرية و الجنس و جرعة الترامادول و مجموع نقاط مقياس الغيبوبة "جلاسكو" و مقياس شدة التسمم و أيضا جرعة النالكزون المستخدمة للعلاج . و فى المقابل لم يكن هناك أي فروق ذات دلالات إحصائية بين مجموعتى الدراسة فى كل من أسباب التسمم بعقار الترامادول أو الفترة الزمنية بين التعرض للتسمم و الوصول للمستشفى أو العلامات الحيوية للمريض أو مستوى تشبع الدم بالأكسجين أو مستوى الغازات بالدم أو مدة العلاج بالمستشفى.

كذلك لوحظ ارتفاع مستوى بروتين S100B بالدم فى مرضى التسمم الحاد بعقار الترامادول مقارنة بالمجموعة الضابطه. كما وجد ارتفاعاً ذو دلالة احصائية فى مستوى بروتين S100B بالدم فى مجموعة المرضي المصابين بتشنجات بعد التسمم الحاد بعقار الترامادول مقارنة بمجموعة المرضي الغير مصابين بتشنجات. وقد أظهرت النتائج أنه عند ارتفاع مستوى بروتين S100B بالدم أكثر من 0,58 ميكروجرام/لتر فإنه يمكن التنبؤ بحدوث التشنجات فى 75% من الحالات بينما يمكن استبعاد حدوث التشنجات فى95,24 % من الحالات عندما يكون مستوى بروتين S100B بالدم أقل من 0,58 ميكروجرام/لتر.

الاستنتاج: يعد مستوى بروتين S100B بالدم مؤشراً جيداً للتنبؤ باحتمال حدوث تشنجات للمرضى المصابين بالتسمم الحاد بعقار الترامادول و بالتالى يمكن استخدامه لتقييم شدة حالة المريض. كما يعتبر الذكور البالغين من الشباب و تناول جرعات زائدة من الترامادول، وزيادة جرعات النالوكزون المستخدمة للعلاج و انخفاض درجة الوعي من العوامل التى تزيد من احتمالية حدوث نوبات التشنج فى حالات التسمم الحاد بعقار الترامادول.

الكلمات المفتاحية: ترامادول – تشنجات – تسمم - بروتين S100B - مؤشر حيوى- عامل التنبؤ