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# Impact of Opium on the Serum Levels of TGF- $\beta$ in Diabetic, Addicted and Addicted-Diabetic Rats

Gholamreza Asadikaram<sup>1,2\*</sup>, Majid Asiabanha<sup>3</sup>, Ahmadreza Sayadi<sup>4</sup>, Abdollah Jafarzadeh<sup>5</sup>, Gholamhossein Hassanshahi<sup>6</sup>

<sup>1</sup>Department of Clinical Biochemistry, Rafsanjan University of Medical Sciences, Rafsanjan, Iran, <sup>2</sup>Department of Clinical Biochemistry, Kerman University of Medical Sciences, Kerman, Iran, <sup>3</sup>Department of Clinical Biochemistry, Qazvin University of Medical Sciences, Qazvin, Iran, <sup>4</sup>Departments of Psychology, <sup>5</sup>Immunology, and <sup>6</sup>Hematology, Rafsanjan University of Medical Sciences, Rafsanjan, Iran

## ABSTRACT

**Background:** Several cells of immune system such as regulatory T cells and macrophages secrete transforming growth factor- $\beta$  (TGF- $\beta$ ) in response to different stimuli. This cytokine has inhibitory effect on immune system and diminished production of this cytokine is associated with autoimmune disorders. **Objective:** The aim of this study was to evaluate the influence of opium addiction on serum level of TGF- $\beta$  in male and female diabetic and non-diabetic Wistar rats. **Methods:** This experimental study was performed on normal, opium addicted, diabetic and addicted-diabetic male and female rats. Serum level of TGF- $\beta$  was measured by ELISA. **Results:** The results of our study indicated that the mean serum level of TGF- $\beta$  in female addicted rats was significantly increased compared to control group ( $p < 0.004$ ). Conversely, in male addicted rats the mean serum level of TGF- $\beta$  was lower compared with control ( $p < 0.065$ ). **Conclusion:** Our results suggest that opium and its derivatives have differential inductive effects on the cytokine expression in male and female rats.

**Keywords:** Addiction, Diabetes, Opium, TGF- $\beta$

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\*Corresponding author: Dr. Gholamreza Asadikaram, Department of Biochemistry, Rafsanjan University of Medical Sciences, Rafsanjan, Iran. Tel: (+) 98 913 1406916, Fax: (+) 98 391 5225209, e-mail: asadi\_ka@yahoo.com

## INTRODUCTION

Several cells of immune system including regulatory T cells and macrophages secrete transforming growth factor- $\beta$  (TGF- $\beta$ ) in response to different stimuli. This cytokine has inhibitory effects on immune system and its decreased level has been demonstrated in autoimmune disorders (1). Opium is a narcotic analgesic drug which is originally obtained from the unripe seed pods of opium poppy. Opium is used as the raw material for the synthesis of some medications such as morphine, noscapine, papaverine and codeine which contain 8-17%, 1-10%, 0.5-1.5% and 0.7-5% opium, respectively (2). Effects of opium on some biochemical parameters have been reported (3,4). Asakura et al, demonstrated that the risk of acute infection is higher in intravenous heroin addicted patients (5). It should be noted that the hemopoiesis is regulated by a complex network of cytokines. Some opium derivatives may interfere with the cytokine network and in turn affect the production of white blood cells (WBC). It has been demonstrated that some opium derivatives can affect the secretion of some cytokines such as IL-2, IL-4, IL-5, IL-10 and IFN- $\gamma$  (6). Morphine increases TGF- $\beta$  production and in this manner results in morphine-induced macrophage apoptosis (7). It also induces the release of TGF- $\beta$  by peripheral blood mononuclear cells (PBMC) (8). Happel et al. demonstrated that D-ala<sup>2</sup>, N-Me-Phe<sup>4</sup>-Gly-ol<sup>5</sup> enkephalin (DAMGO), a highly selective  $\mu$ -opioid agonist, stimulated the expression of TGF- $\beta$  at the protein and mRNA levels (9).

Svetlecic, et al. reported a significant elevation in TGF- $\beta$  level in rats following treatment with papaverine (10). There are however, more than 20 alkaloids (11) and more than 70 components (12) in opium, thus, its effect on cell function may be different from the pure components.

Due to the lack of literature on the effects of opium we examined its effect on the serum level of TGF- $\beta$  in an animal model. In order to study the effects of opium on secretion of this cytokine in different genders, both male and female animals were used in this investigation. Since it is believed by some people that opium possesses some therapeutic properties on various disorders, particularly on diabetes mellitus (3,4), we also included a diabetic group as well.

## MATERIAL AND METHODS

**Materials.** Opium was received from the Police Department. Stroptozocin (Zanosar) was purchased from Pfizer Company (AG, Zurich, Switzerland). Glucose oxidase kit was obtained from Pars azemooon company (Tehran, Iran). TGF- $\beta$  ELISA kit was purchased from ID Labs<sup>TM</sup> Biotechnology Company (London, ON, Canada).

**Animals.** In this experimental study, 28 male and 28 female Wistar rats (250-300 gram) were each divided into 8 groups. Animals were housed on a 12 hours light-dark cycle and had free access to food and water.

**Induction of Diabetes and Addiction.** All animals' procedures were in accordance with the "guide for the care and use of laboratory animal (NIH US publication no.85-23, revised 1985)". Equal number of animals (14 male and 14 female rats) was used for inducing diabetes by injection of stroptozocin at a single dose of 60 mg/kg of the body weight intraperitoneally. After 3 days, 0.5 ml blood was obtained from orbit cavity by thin heparinized tubes, plasma glucose was measured by the glucose oxidase method

and the animals with a plasma glucose level more than 200 mg/dl were regarded as diabetic. Seven male and 7 female diabetic, 7 male and 7 female non-diabetic animals were treated with twice daily opium dissolved in saline for 8 consecutive days (8 AM and 8 PM) by peritoneal injection. The control group received only normal saline as a vehicle. The protocol of treatment with opium was as follows: 1st day, 30 mg/kg; 2nd day, 60 mg/kg; 3rd day, 90 mg/kg; 4th day, 120 mg/kg; 5th day, 150 mg/kg; and the 6th day, 150 mg/kg. The animals were treated with opium on the 7<sup>th</sup> and 8<sup>th</sup> day as the 6th day 150 mg/kg was the maximum tolerable dose for these animals. Withdrawal signs in opium-dependent rats were lacrimation, piloerection, salivation, an ejaculate-like discharge, irritability, hyperactivity and wet-dog shakes. At the end of treatment protocol (day 9<sup>th</sup>), a single dose of 150 mg/kg opium was injected and after 3 hours, the animals were anesthetized with ether and blood was collected. Animals were humanly killed after blood collection. Serum was separated and TGF- $\beta$  was determined.

**Determination of the Serum Level of TGF- $\beta$ .** Serum level of TGF- $\beta$  was determined using commercial ELSA kit (ID Labs<sup>TM</sup> Biotechnology).

**Statistical Analysis.** Serum TGF- $\beta$  concentrations were expressed as mean  $\pm$  S.D. Data were analyzed using the Mann-Whitney test. A probability level of  $p < 0.05$  was considered significant.

## RESULTS

Results of our study indicated that the mean value of TGF- $\beta$  in addicted-diabetic female rats ( $57.20 \pm 50.86$  pg/ml) was higher than that of the non-addicted-diabetic female group and a tendency to a significant difference was observed ( $17.78 \pm 12.53$  pg/ml) ( $p = 0.061$ , Table 1).

**Table 1. Concentration of TGF- $\beta$  in addicted-diabetic (case) and diabetic (control) male and female rats. Data are from 7 animals is presented in each group .**

Serum cytokine	Male		P value	Female		P value
	Case	Control		Case	Control	
TGF- $\beta$ (pg/ml) (mean $\pm$ SD)	28.63 $\pm$ 35	32.63 $\pm$ 28.5	0.940	57.20 $\pm$ 50.86	17.78 $\pm$ 12.53	0.061

The results showed that level of TGF- $\beta$  in male addicted animals was slightly lower than the control ( $11.90 \pm 6.68$  and  $20.00 \pm 3.79$  pg/ml,  $p = 0.065$ , respectively), while in female addicted animals, serum TGF- $\beta$  significantly increased in comparison to the control group ( $20.67 \pm 3.27$  and  $15.10 \pm 0.93$  pg/ml,  $p = 0.004$ , respectively) (Table 2).

**Table 2. Concentration of TGF- $\beta$  in addicted (case) and non-addicted (control) male and female rats. Data are from 7 animals is presented in each group.**

Serum cytokine	Male		P value	Female		P value
	Case	Control		Case	Control	
TGF- $\beta$ (pg/ml) (mean $\pm$ SD)	11.90 $\pm$ 6.68	20.00 $\pm$ 3.79	0.065	20.67 $\pm$ 3.27	15.10 $\pm$ 0.93	0.004

However, in male addicted animals the mean serum level of TGF- $\beta$  was significantly lower compared with that of female addicted animals ( $p < 0.015$ ), while in female non-addicted animals, the level significantly increased compared to male non-addicted animals ( $p < 0.009$ ) (Table 3).

**Table 3. Comparison of serum TGF- $\beta$  concentration in addicted (case) and non-addicted (control) male and female rats. Data are from 7 animals is presented in each group.**

Serum cytokine	Case		P value	Control		P value
	Male	Female		Male	Female	
TGF- $\beta$ (pg/ml) (mean $\pm$ SD)	11.90 $\pm$ 6.78	20.67 $\pm$ 3.27	0.015	20.00 $\pm$ 3.80	15.10 $\pm$ 0.93	0.009

No significant difference was observed between the level of TGF- $\beta$  in addicted-diabetic and non-addicted-diabetic male and female rats (Table 4).

**Table 4. Concentration of TGF- $\beta$  in addicted-diabetic and addicted-non-diabetic male and female rats. Data are from 7 animals is presented in each group.**

Serum cytokine	Male		P value	Female		P value
	Diabetic Addicted	Non-diabetic Addicted		Diabetic Addicted	Non-diabetic Addicted	
TGF- $\beta$ (pg/ml) (mean $\pm$ SD)	28.63 $\pm$ 35.06	11.40 $\pm$ 6.78	0.62	57.87 $\pm$ 50.86	20.67 $\pm$ 3.26	0.18

Similarly, no significant difference was observed between the level of TGF- $\beta$  in addicted-diabetic and non-addicted diabetic males and females (Table 5), and also the diabetic and non-diabetic male and female rats (Table 6).

**Table 5. Comparison of TGF- $\beta$  concentration in addicted-diabetic (case) and diabetic control male and female rats. Data are from 7 animals is presented in each group.**

Serum cyto- kine	Case		P value	Control		P value
	Male	Female		Male	Female	
TGF- $\beta$ (pg/ml) (mean $\pm$ SD)	28.63 $\pm$ 35.06	57.20 $\pm$ 50.86	0.161	32.63 $\pm$ 28.51	17.78 $\pm$ 12.53	0.491

**Table 6. Concentration of TGF- $\beta$  in diabetic and non-diabetic male and female rats. Data are from 7 animals in each group.**

Serum cyto- kine	Male		P value	Female		P value
	Diabetic	Non-diabetic		Diabetic	Non-diabetic	
TGF- $\beta$ (pg/ml) (mean $\pm$ SD)	32.63 $\pm$ 28.51	20.00 $\pm$ 3.80	0.69	17.78 $\pm$ 12.53	15.10 $\pm$ 0.93	0.95

## DISCUSSION

The results of this study showed that the mean serum level of TGF- $\beta$  in female rats was significantly higher than that of the male ones. The elevated level of TGF- $\beta$  in female animals compared to the male is in accordance with the weaker immune system of this gender. Accommodating data revealed that male animals are more susceptible to bacterial, viral and fungal infections in comparison to females (13). The mortality rate of men due to infectious diseases is also more than women (14). Accordingly, the rate of autoimmune diseases such as multiple sclerosis, rheumatoid arthritis and systemic lupus erythmatosis is higher in women (15). It is also reported that the pattern of cytokine expression is different in males and females and this in a way influences the sensitivity or resistance to diseases (16).

We also observed that exposure to opium leads to a slight decrease of TGF- $\beta$  in male rats and a significant increased level of this cytokine in female rats. The main cause of the altered TGF- $\beta$  level in different genders is not as yet clear but it seems that opium and/or its derivatives may have different inductive effects on the hormone and cytokine expression in the male and female rats. For example, *in vitro* studies demonstrated that morphine, the main opium alkaloid, causes increased LH secretion from hypophysis of a kind of male fish while it has not affected the female fish. Also, altoroxan, an agonist of an opioid receptor, caused a decrease in LH secretion in male mice and increased its level in female mice (17). These observations show that probably the expression of opioid receptors or their signal transduction pathways are gender-dependent. In fact the effects of opium and its derivatives on regulation of hormones and production of cytokines may be different in different genders. In this study we also examined the association between serum level of TGF- $\beta$  with the presence of apoptosis in different tissues of male and female addicted rats (data not shown). *In vitro* studies have demonstrated that

morphine induces apoptosis in macrophages via TGF- $\beta$  production. TGF- $\beta$  leads to apoptosis by autocrine and paracrine pathways which could be inhibited by anti-TGF- $\beta$  antibody (7). Our results also indicated that the rate of apoptosis in female addicted animals increased with enhanced TGF- $\beta$  production (data not shown). Interestingly, in male addicted rats, the increase in brain cell apoptosis is reversely related to TGF- $\beta$  level. On the other hand, our observations indicate that the rate of apoptosis in male addicted rat liver cells is less than females (unpublished data). Based on these results, it could be assumed that in male addicted rats, apoptosis of liver cells is not associated with the level of TGF- $\beta$ . It has been shown that some opium derivatives, such as morphine and papaverine, had increasing effect on TGF- $\beta$  production (7,8,10). Increasing effect of opium in female is in parallel with these reports, but in male animals our results disagree with previous reports.

TGF- $\beta$  level increased in the diabetic animals (18,19,20) due to an increase in glucose level and followed by glycation of the end products of PKC and MAPK pathway (20). In female animals our results are in agreement with previous studies since serum TGF- $\beta$  level in addicted-diabetic animals was higher than that of the addicted-non-diabetic ones (Table 5). Although, TGF- $\beta$  concentration was higher in diabetic compared with non-diabetic animals in both sexes, no significant difference was observed between the groups (Table 6).

It has been reported that morphine addiction induces the production of several other cytokines such as IL-6, and TGF- $\alpha$  in peripheral blood mononuclear cells (8). It has been demonstrated that TGF- $\alpha$  has apoptotic properties, whereas IL-6 is an anti-apoptotic cytokine (21,22). Due to the different profiles of the production of these two cytokines in male and female animals (16,23) and also due to various responses of different tissues to these cytokines, it seems that probably addiction induces apoptosis via different pathways in the two genders. It has been demonstrated that sex hormones influence apoptosis; testosterone induces and progesterone inhibits apoptosis (24,25). In a previous study we showed that some other biochemical parameters are gender dependent in addicted subjects (3,4). Therefore, it is possible that the inductive effects of opium and its derivatives on apoptosis are indirect and through their effects on the secretion of sex hormones in different genders. Overall, our results showed that consuming opium leads to a decreased level of serum TGF- $\beta$  in the male and increased level in female rats. The main cause of the altered TGF- $\beta$  level in different genders is not clear, but it seems that opium and its derivatives have different inductive effects on cytokine expression in the male and female rats.

## ACKNOWLEDGMENTS

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