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Evaluation of Circulating Immune Complexes and Anti-phospholipid Antibodies (anti Beta 2 Glycoprotein 1) in Heroin Addicts and their Clinical Significance

Natasa Simonovska¹, Andon Chibishev¹, Cvetanka Bozinovska¹, Ladislava Grcevska², Koco Dimitrovski³, Violeta Neceva³

University Clinic of Toxicology, Clinical Centre, Skopje, R. Macedonia¹

University Clinic of Nephrology, Clinical Centre, Skopje, R. Macedonia²

Institute of Transfusiology, Clinical Centre, Skopje, R. Macedonia³

Introduction. Earlier studies have reported that heroin might cause the structural and antigen changes on numerous tissues, organs and subsequent development of autoimmune reactions (production of antibodies and creation of immune complexes) as a result the immunotoxic effect of heroin. **The aims** of our study were to: a) Evaluate CIC and antiβ2GP1 in heroin addicts; b) Correlate between the values of the obtained CIC and antiβ2GP1 (stratified by the duration and route of heroin application); c) Compare the CIC and antiβ2GP1 in heroin addicts and the control group and d) Assess the clinical importance of CIC and antiβ2GP1 in heroin addicts. **Patients and methods.** This was a cross-sectional study performed at the University Clinic of Toxicology and the Institute of Transfusiology, Skopje, Republic of Macedonia. Patients referred to the Clinic for clinical examinations who met the inclusion criteria were analyzed. Protocol for work was the following: 1.) detailed anamnestic data, 2.) a whole set of laboratory biochemical blood and urine analyses, 3.) examination with the Schiller's twelve-channel ECG; 4.) toxicological analyses for opioids in a urine sample; circulating immune complexes and 5.) antiphospholipid antibodies (antiβ2GP1, fractions: IgA, IgG, IgM). The obtained results were statistically analyzed. **Results.** We included 37 heroin addicts and a control group of 27 healthy subjects. Male abusers predominated over female in - 28 (76%) subjects; mean age being 26 ± 5.06. The results which refer to the increased values of circulating immune complexes have shown a high statistically significant dominance of heroin addicts, in comparison with the control group ($p < 0.01$) and increased values above the reference ones of IgG antiβ2GP1, alone in the group of intravenous heroin abusers ($p < 0.025$). The mean duration of the heroin use in intravenous abusers was 6.21 ± 3.25 years, whereas in those snorting heroin was 5.15 ± 2.26 years. Duration of heroin application was in a positive correlation with IgG antiβ2GP1 ($p = 0.35$). **Conclusions.** Our data showed that heroin-dependent patients in our study had increased values of circulating immune complexes and changes in IgG and IgM antiβ2GP1 with significantly increased values of IgG antiβ2GP1 in the intravenous heroin abusers. The duration of heroin application is in direct proportional relationship with IgG antiβ2GP1. Heroin addicts had significantly higher values of circulating immune complexes and statistically significant difference in IgG antiβ2GP1, in comparison with the control group. Changes in the fractions of antiβ2GP1 and CIC suggest a possible relation with the somatic changes found in heroin addicts (i.e. thrombocytopenia, reduced renal clearance, etc). **KEY WORDS:** HEROIN ADDICTS, CIRCULATING IMMUNE COMPLEXES, ANTIPHOSPHOLIPID ANTIBODIES (ANTIβ2GP1)

1. INTRODUCTION

The need for medical help to heroin addicts is increasing, not only as a result of an acute intoxication with opioids, but also as a result of a large number of somatic changes (kidney diseases, vascular disorders, etc.). The immunomodulatory effect of opioids was initially demonstrated in 1890 when Cantacuzene emphasized the effect of opioids on cellular immune suppression and decreased resistance to bacterial infection in guinea pigs treated with morphine (1). An earlier study have confirmed that heroin and its diluents might cause structural and antigen changes on numerous tissues and organs by subsequent development of the autoimmune reactions (e.g. antibody production and formation of immune complexes) resulting from heroin immunotoxic effect (2). The immunomodulatory effect of heroin and its diluents has been described to be the result, either of the direct effect of immune cells receptors or indirectly by the similar receptors of the nervous system cells (2). Different autoantibodies and immunologic abnormalities have been described in the heroin addicts. The incidence of autoantibodies and the severity of clinical symptoms are related to the duration of drug addiction and the titer of antibodies is not always related to the HIV presence or chronic viral infection. It is confirmed that the titer of antibodies is reduced after the cessation of the heroin abuse (3).

Corresponding author: Andon Chibishev, MD, PhD. University Clinic for Toxicology, Clinical Centre, Vodnjanska 17, 1000 Skopje, R. of Macedonia. Tel: +389 2 3211 072. +389 2 3237 504. E-mail:toksikourgentna@gmail.com

The most common observed auto-antibodies in this population were: 1.) rheumatoid factor, 2.) anticardiolipin antibodies, 3.) antinuclear antibodies (ANA), 4.) antiphospholipid antibodies (anti β 2GP1) and 5.) some other changes in the immune system (4). Antiphospholipid antibodies are a heterogeneous group of autoantibodies (i.e. a family of autoimmune and alloimmune immunoglobulins IgG, IgA, IgM or their mixture). It is a dilemma, whether they cause thrombosis, appear as a result of thrombosis or do appear accidentally. Hence, their eventual association with vascular complications in heroin addicts is yet unclear (3, 5, 6).

As a result of changes developed in the immune system, during application of heroin and its diluents, some studies have reported the elevated circulating immune complexes titer thus attempting to highlight some conditions, which appear in heroin abusers, such as: 1.) thrombocytopenia, 2.) kidney disorders, etc. Circulating immune complexes (CIC) appear as a result of binding of an antibody to a soluble antigen. They are a constituent part of: the autoimmune diseases, SLE, cryoglobulinemia, rheumatoid arthritis, scleroderma, Sjogren's syndrome, etc. A correlation between CIC and clinical manifestations affecting the kidneys, heart and skin has been described (4). Assessment of the heroin addicts referred to our Clinic of Toxicology revealed somatic changes, which were the motive for evaluation of certain segments in the immunologic response of this group of patients and their possible interrelationship by explaining certain clinical conditions.

The aims of our study were to: a) Evaluate CIC and anti β 2GP1 in heroin addicts; b) Correlate between the values of the obtained CIC and anti β 2GP1 (stratified by the duration and route of heroin application) c) Compare the CIC and anti β 2GP1 in heroin addicts and the control group; d) Assess the clinical importance of CIC and anti β 2GP1 in heroin addicts.

2. PATIENTS AND METHODS

This was a cross-sectional study performed at the University Clinic of Toxicology and the Institute of Transfusiol-

ogy in Skopje. Patients referred to the Clinic for clinical examinations were analyzed. Inclusion criteria for the study were the following: 1.) history of heroin abuse and 2.) confirmation of heroin abuse with toxicological analysis of the urine. Exclusion criteria was the previous history of autoimmune and infectious diseases. Data for subjects were taken from previously prepared questionnaire, along with a whole set of laboratory biochemical parameters [blood (10 ml)] and urine analyses. Additionally, an estimated creatinine clearance rate (eC_{Cr}) using Cockcroft-Gault formula was utilized together with the examination with the Schiller's twelve-channel ECG and toxicological urine analyses for opioids (i.e. fluorescence polarization immunoassay technique – FPIA) (7) (Institute of the Forensic Medicine, Skopje) used: a) the circulating immune complexes: b) spectrophotometric method for determination of the concentration of circulating immune complexes; c) apparatus: spectrophotometer of 740 wave-length (Institute of Transfusiology, Skopje); d) antiphospholipid antibodies (e.g. anti β 2GP1, fractions: IgA, IgG, IgM with, ELISA method:) (9) (At the Institute of Transfusiology, Skopje) a circulating immune complexes and anti β 2GP1 were examined in a blood sample (5 ml). Subjects participating in this study gave their written informed consent.

The statistical analysis of the results was performed by the descriptive and analytical statistical methods. Descriptive methods included: 1.) the arithmetic mean; 2.) standard deviation; 3.) frequency and 4.) cross tabulations. For testing of the null hypothesis, the following analytical statistical tests were used: 1.) Fischer's exact test, 2.) Mann-Whitney U-test of inversion and 3.) Spearman's coefficient of non-parametric correlation. Probability levels (p) were p=0.05 and p=0.01. The entire statistical analysis was done with the statistical program SPSS, version 14.00, Chicago, IL.

3. RESULTS

We included 37 heroin addicts; where the male abusers were predominant with - 28 (76%) subjects and the mean age of 26 \pm 5.06 y. The youngest abuser was 19 years old and the oldest 47 years. The control group consisted of 27; (i.e. age - and sex-matched healthy subjects). The examined immunologic parameters in the control group confirmed their reference values as per healthy population. Out of the total number of examined heroin addicts, 24 injected heroin intravenously and 13 by snorting. The mean duration of heroin use in intravenous abusers was 6.21 \pm 3.25 years, whereas, in those snorting heroin it was 5.15 \pm 2.26 years. The du-

Examined parameters n=37	n	p	Associated cl. conditions
CIC	21/37	p<0.01	Thrombocytopenia n=2 X=62,5x10 ⁹ /l; Proteinuria n=11 X=0,32g/l; decreased kidney clearance n=3 GFR=75ml/min ;
anti β 2GP1 IgA	/	p>0.05	/
anti β 2GP1 IgG	10/37	p<0.025	Vascular changes
anti β 2GP1 IgM	2/37	p>0.05	/

TABLE 1. Heroin addicts with increased values of CIC and anti β 2GP1 fractions: IgA, IgG, IgM (Fischer's exact test) and associated clinical conditions.. CIC (circulating immune complexes), anti β 2GP1 (anti-beta 2 glycoprotein 1), n (number of subjects), proteinuria ref.v: 0,lg/l; Trombocytopenia ref.v: 140-340 x10⁹/l

	CIC		Anti B2GP1 IgA		Anti B2GP1 IgG		Anti B2GP1 IgM	
	n	X \pm SD	n	X \pm SD	n	X \pm SD	n	X \pm SD
i/v	18	0.10 \pm 6.2	0	/	10	25.6 \pm 3,53	2	21.0 \pm 0.0
Snor.	03	0.07 \pm 2.3	0	/	0	/	0	/
p	p>0.05		p>0.05		p<0.025		p>0.05	

TABLE 2. Serum concentration of relevant immunologic parameters (CIC and anti β 2GP1 fractions: IgA, IgG, IgM, for values above the control ones), according to the route of heroin application. CIC (circulating immune complexes, ref. v.: 0.00-0.05 g/l), anti β 2GP1 (anti-beta 2 glycoprotein 1) fractions: IgA, IgG, IgM (ref. v.: 0-20 SAU), n (number of subjects); X (mean value); SD (standard deviation); i/v (intravenous application); snor. (snorting)

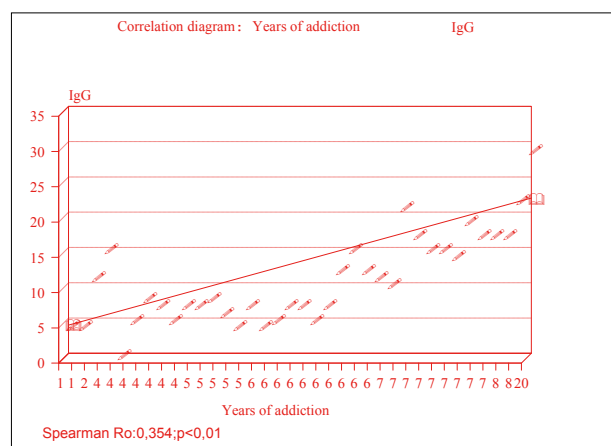


FIGURE 1. Correlation diagram (years of addiction)

ration of heroin application positively correlated with IgG anti β 2GP1.

4. DISCUSSION

As reported in the literature, the heroin and its diluents may cause the immunologic abnormalities, in heroin addicts, as reflected as the presence of different autoantibodies. By reporting their experiences, the authors emphasize the clinical importance of these immunologic changes, in heroin addicts and at the same time they give a possible explanation about some of the somatic changes that appear. Crowe et al. stated that kidney complications might develop in heroin addicts, but their pathogenesis is still unclear (10). Earlier studies reported that heroin or some of its diluents acted as an antigen, leading to renal deposition of immune complexes in the kidney (10). Recent studies have shown that morphine may have a direct effect on the glomerulus, causing proliferation of fibroblasts and a decreased degradation of type IV collagen (10). Manner et al., in his study presented his experience of finding renal amyloidosis in heroin addicts, as verified by the renal biopsy and suggested that one should always have in mind the renal amyloidosis in chronic heroin addicts with proteinuria and renal impairment (11, 12). Similar experience was published by Connolly et al., in his study (13). In our group of 37 heroin addicts, 21 had increased values of circulating immune complexes above the control values. In three of them, a reduced renal clearance was registered, whereas in 11 proteinuria was observed. Savona et al., described an autoimmune thrombocytopenia, in heroin-dependent in-

dividuals, thus suggesting a new immunologic disorder in the blood cells. Elevated circulating immune complexes were noticed in 33 patients (14). Moss et al., pointed out that heroin may be a possible cause for immune thrombocytopenia and he anticipated that platelets may be rapidly removed from the circulation, as a result of the attached

“drug-antibody” immune complexes (15). In our group of 21 heroin-addicted subjects with elevated values of circulating immune complexes above the reference level, thrombocytopenia was observed only in 2 patients.

Analysis of anti β 2 GP1 was also performed in our examined group. Increased values above the reference levels were detected in the IgG fraction, thus revealing that two of the patients had thrombosis of the legs in the past. In one of them, the increased size of right lower limb was noticed and the thorough examination excluded the thrombosis and other evident vascular disorders. Eight months later, the follow-up assessment of these patients detected thrombosis in the left lower limb in one of them. Nikolova et al., in her study of 10 patients found a high ANCA titer in two, elevated ANA titer in four (e.g. two of whom were antiHCV positive) and positive IGM anticardiolipin antibodies in 3 heroin addicts (i.e. two of whom had biopsy-proven chronic tubulo-interstitial nephritis) (3). Four patients in the same study had elevated titer of antiphospholipid antibodies (anti β 2GP1). Such elevated anti β 2GP1 titers were described by several authors, in association with arterial or venous thrombosis or without thrombotic changes (3). It has been reported that IgG anti β 2GP1 is closely associated with venous and arterial thrombosis (16). Over a longer follow-up of these patients or after the cessation of heroin abuse these antibodies have been removed. Nikolova et al., reported that the autoantibody incidence correlate with the duration of heroin addiction (3). Our results have confirmed

this positive correlation with the duration of heroin application for IgG anti β 2GP1, alone.

5. CONCLUSION

Our data showed that heroin-dependent patients, in our study, had increased values of circulating immune complexes and changes in IgG and IgM anti β 2GP1, with significantly increased values of IgG anti β 2GP1 in the intravenous heroin abusers. The duration of heroin application is in direct proportional relationship with IgG anti β 2GP1. Heroin addicts had significantly higher values of circulating immune complexes and statistically significant difference in IgG anti β 2GP1 in comparison with the control group. Changes in the fractions of anti β 2GP1 and CIC suggest a possible relation with the somatic changes found in heroin addicts (thrombocytopenia, reduced renal clearance, vascular disorders, etc.).

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