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Identification of humoral immune indicators in some clinical manifestations in patients with heroin use disorder without hepatitis C infection

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Summary

Background: The effect of opioids on the immune system is a complex phenomenon dependent on such variables as the type of opioid, the character of the response (humoral versus cellular) and the types of cells involved. **Aim:** To characterize humoral immune indicators and determine their predictive impact on the onset of common clinical manifestations observed in heroin users without hepatitis C infection. **Methods:** A total of 140 outpatients were enrolled in this cross-sectional study, which lasted over a 3.5-year period at the University Clinic of Toxicology in Skopje from January 2009 to June 2012. Multivariate logistic regression analysis was used to determine the impact of several humoral immune indicators at the onset of the common clinical manifestations. The following instruments were used for analysis of patient samples: immunoturbidimetric assay, indirect immunofluorescence, spectrophotometer, fluorescence polarization immunoassay. **Results:** Most of the patients were male n=108, with an average age of 28.34 ± 5.34 years. Arthralgia was observed in 42.14%, skin changes in 12.14%, respiratory difficulties in 15.0%, neurological disorders in 12.86%, Raynaud's disease in 19.28%, and proteinuria in 42.86% of patients. Multivariate logistic regression analysis showed that significant factors having a potential correlation with intravenous heroin usage included: immunoglobulin G (0.027), immunoglobulin M (0.026), and cryoglobulins (<0.001). **Conclusions:** Heroin users with cryoglobulinemia have a greater chance of developing arthralgia, skin changes, respiratory difficulties, neurological disorders, and Raynaud's disease. Some of these conditions were more common among participants who were taking heroin intravenously. These manifestations require treatment that is delivered on time.

Key Words: Heroin use disorder; hepatitis C; virus negative; clinical manifestations; humoral immune indicators

1. Introduction

The effect of opioids on the immune system is a complex phenomenon dependent on such variables as the type of opioid, the character of the response (humoral versus cellular) and the types of cells involved [2]. Humoral immunity is mediated by antibodies produced by cells that have a B lymphocyte lineage [7]. The idea that exogenous opioids can affect the immune functions was originally suggested by Cantacuze, in 1898. Exogenous opioids appear to elicit various modulatory profiles on immune function. Our current understanding of the interaction between opioids and the immune system is based on pharmacological studies, and several mechanisms have been proposed. Additionally, in vitro experiments suggest that opioids act directly on immune cells [4]. Several studies have shown that intravenous heroin users have abnormal immune functions, notably an increase in immunoglobulins and lymphocytes, a decreased mitogen response from lymphocytes in culture, and an abnormal rosette formation of T lymphocytes [1]. A number of studies have suggested that heroin and its diluents cause structural and antigen changes in numerous tissues and organs followed by the subsequent development of autoimmune reactions (the production of antibodies and the creation of immune complexes) [8]. Through a cross-sectional assessment

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of peripheral blood B cells and plasma analytes we have identified substantial alterations in intravenous drug users (IDUs) that are associated with increased systemic inflammation, and which have the potential to influence the quality of the B cell and antibody response to vaccinations and infections, and contribute to comorbidities associated with inflammation [6].

It is unclear whether these abnormalities are due to a direct effect of opioids, especially if these are taken in fluctuating quantities, to the various contaminants that are found in illegal street heroin, or to other factors [1]. In addition to increased exposure to pathogens, prolonged intravenous drug use has been associated with immune dysregulation that can diminish effective innate and adaptive immune responses. The impact of intravenous drug use on humoral responses is poorly defined. We sought to identify potential differences in the humoral profile of intravenous drug users (IDUs) that could impact their immune response and overall health [6].

The hypothesis of this study was that some humoral immune indicators have predictive impact on the onset of common clinical manifestations observed in patients with heroin use disorder but without hepatitis C infection.

Aim: The purpose of this study was to characterize the humoral immune indicators: immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), complement components (C3, C4), rheumatoid factor (RF), circulating immune complex (CIC), antinuclear antibodies (ANA), cryoglobulins, and determine their predictive impact on the occurrence of skin changes, Raynaud phenomena, respiratory difficulties, neurological disorders, and proteinuria, in this population.

2. Methods

2.1. Design of the study

This was a cross-sectional study that included a total of 140 patients with heroin use disorder. A total of 140 outpatients, each with a Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (DSM-IV) diagnosis of heroin use disorder, was examined at the University Clinic of Toxicology in Skopje over a 3.5-year period, from January 2009 to June 2012. These individuals were consecutive patients who visited our clinic, for one of several reasons: clinical examination despite continuing heroin use, rapid detoxification, or to begin treatment for heroin use disorder, with buprenorphine. All participants underwent an interview and a complete clinical examination performed by University Clinic of Toxicology specialists in internal medicine. Patients were examined for the presence of any physical manifestations (e.g. musculoskeletal, cardiovascular, gastrointestinal, respiratory, neurological, hormonal, metabolic disorders, and skin changes). A control group consisted of asymptomatic patients with heroin use disorder. This study was carried out with the approval of, and the written consent obtained from the patients who participated in this study.

During the first visit, blood and urine samples were collected from all patients to measure immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), complement components (C3, C4), rheumatoid factor (RF), circulating immune complexes (CIC), antinuclear antibodies (ANA) and cryoglobulins. Urine samples were used to assess opioid and protein levels. The most recent use of heroin was evaluated according to the history of heroin use disorder, physical examination, positive toxicological screening for opioids, and clinical observations.

2.2. Sample

The study began with 223 subjects, of whom 83 declined to participate in the investigation. The patients who declined to participate had socioeconomic, demographic and clinical features comparable with those that participated in this study.

The following inclusion criteria were applied: a current diagnosis of heroin use disorder, according to the criteria set out in the Diagnostic and Statistical Manual of Mental Disorders (4th ed.) (DSM-IV), and a positive toxicological screening result for opioids. Exclusion criteria were as follows: hepatitis B surface antigen positivity, hepatitis C virus or human immunodeficiency virus seropositivity, tuberculosis, signs and symptoms of an infectious syndrome, any previous comorbid autoimmune condition, positive toxicological findings for methadone, benzodiazepines, tramadol, cocaine, or amphetamines, concurrent therapy for acute or chronic disease, or evidence of active opioid replacement treatment.

2.3. Instruments

The following instruments were used for testing: Cobas-Integra 700 (Roche, Basel, Switzerland), immunoturbidimetric assay (quantitative determination of immunoglobulin A (IgA), immunoglobulin

Table 1. Demographic characteristics (gender, route of administration, age, duration of heroin use disorder) in asymptomatic and symptomatic patients with heroin use disorder

N=140		N(%)	Asymptomatic n=33 N(%)	Symptomatic n=107 N(%)	P value
Gender	male	108(77.14)	22 (66.67)	86 (80.37)	p=0.1 *(Chi-square test)*
	female	32(22.86)	11 (33.33)	21 (19.63)	
Route of administration	intravenously	88(62.85)	19 (57.58)	69 (64.49)	p=0.47 *(Chi-square test)*
	inhalation	52(37.15)	14 (42.42)	38 (35.51)	
Age	mean ± SD		24.88 ± 4.9	29.42 ± 5.4	*p<0.01 (Student t-test)*
Duration of heroin use disorder	mean ± SD		5.39 ± 4.5	9.49 ± 4.6	*p<0.01 (Student t-test)*

N-number of patients

G (IgG), immunoglobulin M (IgM), complement components (C3,C4), rheumatoid factor (RF); fluorescence, indirect immunofluorescence the determine the presence of antinuclear antibodies HEp-2 (ANA-HEp-2); spectrophotometer for the determination of circulating immune complexes (CIC); fluorescence polarization immunoassay (FPIA) to determine the presence of opioids in urine samples; cryoglobulin: qualitative method according to a reference method (to evaluate serum cryoglobulins, blood specimens were collected in warm tubes (37°C) in the absence of anticoagulants. An aliquot of 15-20 mL blood was collected. The serum was kept warm until the cells were separated in the laboratory. The serum was kept at 4°C for one to seven days. If clumping was observed, cryoglobulins were present. Negative tests were confirmed after seven days); proteinuria Cobas Integra 400, turbidimetric assay: Cobas Integra, and total protein urine test. All tests were performed at the Institute of Clinical Biochemistry, the Institute of Forensic Medicine, and the Institute of Transfusion Medicine, in Skopje.

2.4. Ethical approval:

This study was carried out with the approval of the Ethics Committee of the Medical Faculty, Skopje (date: 26.06.2008; 03-2046).

2.5. Data analysis

Statistical analysis was conducted using the statistical program SPSS, version 13.0 (SPSS, Inc., Chicago, IL, USA). The following statistical methods were used: descriptive methods, Chi-square test, Fisher's exact two-tailed test, Student t-test and Mann-Whitney test. Univariate logistic regression analysis was used to identify significant factors having a potential correlation with intravenous heroin usage. Multivariate logistic regression analysis was used to determine the impact of several immune factors as significant independent predictors of the onset of some clinical manifestations in heroin users without hepatitis C infection. The risk factors were quantified by calculation of risks with odds ratio (OR) and by the Confidence intervals (Cl-95%). The statistical significance was defined as a P-value less than 0.05.

The normality of data distribution was tested with Kolmogorov-Smirnov and Lillefors test for normality (Skewness and Kurtosis measures).

3. Results

The majority of patients were male n=108 (77.14%). The average age was 27.15 ± 5.15 years. Most patients administered heroin intravenously n=88 (62.85%) while the remaining 52 utilized an inhalation route of administration. The average duration of heroin use disorder was 7.44 ± 4.55 years, with an average onset at age 18. Regarding the duration of heroin use disorder, participants were divided into three groups: from 0 to 3 years n=28, 4 to 7 years n=34, and more than 7 years n=78. There was no significant difference between asymptomatic and symptomatic patients with heroin use disorder with respect to gender (p=0.1) or route of administration (p=0.47). The symptomatic group of patients were significantly older (p<0,001) and with a longer duration of heroin use disorder in comparison with the asymptomatic group (p<0,001) (Table 1).

Among all patients, n=33 (23.57%) had no symptoms, n=46 (32.86%) patients had one symp-

Table 2. The most frequent clinical manifestations in patients with heroin use disorder

Clinical manifestations	N=140	Route of administration		Duration of heroin use disorder (years)		
	(N/%)	intravenously	inhalation	0-3	4-7	>7
Arthralgia	59(42.14)	43(72.9)	16(27.1)	6(10.17)	11(18.64)	42(71.19)
Skin changes	17(12.14)	13(76.47)	4(23.53)	/	1(5.88)	16(94.12)
Respiratory difficulties	21(15.00)	17(80.95)	4(19.05)	3(14.28)	2(9.52)	16(76.19)
Neurological disorders	18(12.86)	14(77.77)	4(22.23)	1(5.55)	7(38.89)	10(55.55)
Raynaud phenomenon	27(19.28)	22(81.48)	5(18.52)	1(3.70)	7(25.93)	19((70.37)
Proteinuria	60(42.86)	38(63.33)	22(36.67)	9(15.00)	9(15.00)	42(70.00)

N-number of patients

tom, n=34 (24.28%) patients had two symptoms, n=19 (13.57%) patients had three, n=5 (3.57%) patients had four, n=2 (1.43%) patients had five, and one patient (0.71%) had six symptoms. The most frequent combination of symptoms was a combination of arthralgia, Raynaud phenomenon, and proteinuria n=17 (12.14%) (Table 2)

This study showed significantly increased mean values of IgG fraction (p=0,004), C3 component (p=0,001) and CIC (p=0,003) in the symptomatic group compared to the asymptomatic group (Table 3)

Significantly increased values above reference levels for IgA fraction (p=0.038), CIC (p=0.014) and cryoglobulins (p<0,001) were observed in the symptomatic group of patients with heroin use disorder.

Univariate logistic regression analysis showed that significant factors having a potential correlation with intravenous heroin usage included: duration of heroin use disorder (0.005), IgG fraction (0.002), IgM fraction (0.005), RF (0.033), and cryoglobulins (0.001). Multivariate logistic regression analysis showed that significant factors having a potential correlation with intravenous heroin usage included: IgG fraction (0.027), IgM fraction (0.026), and cryoglo-

Table 3 Mean values of serum autoimmune markers (IgA, IgG, IgM, C3, C4, RF, CIC) in asymptomatic and symptomatic patients with heroin use disorder

		mean ± SD	min - max	p value
IgA (0.7-4.0g/l)	asymptomatic	2.18 ± 0.75	1.05 – 4.23	p=0.06
IgG (7.0-16.0g/l)	asymptomatic	12.07 ± 2.85	7.72 – 18.96	p<0.01
	symptomatic	13.25 ± 4.01	9.19 – 24.64	
IgM (0.4-2.3g/l)	asymptomatic	1.29 ± 0.6	0.36 – 2.6	p=0.82
	symptomatic	1.79 ± 0.9	0.46 – 3.32	
C3 (0.8-1.4g/l)	asymptomatic	1.03 ± 0.3	0.41 – 1.62	p<0.01
	symptomatic	1.32 ± 0.3	0.83 – 1.87	
C4 (0.2-0.5g/l)	asymptomatic	0.21 ± 0.08	0.06 – 0.34	p=0.49
	symptomatic	0.26 ± 0.09	0.11 – 0.46	
RF (<13IU/ml)	asymptomatic	8.43 ± 3.4	1.2 – 14.8	p=0.09
	symptomatic	9.95 ± 4.7	0.1 – 22	
CIC (0.00-0.05 g/l)	asymptomatic	0.04 ± 0.02	median(IQR) 0.04 (0.04 – 0.05)	p<0.01
	symptomatic	0.07 ± 0.12	median(IQR) 0.05 (0.04 – 0.08)	

(Student t-test) p(Mann-Whitney test)
IgA-immunoglobulin A, IgG-immunoglobulin G, IgM-immunoglobulin M, C3-complement components 3, C4-complement components 4, RF-rheumatoid factor, CIC-circulating immune complexes

Table 4 Univariate and multivariate logistic regression analysis to identify significant factors with potential correlation with intravenous heroin usage

Univariate logistic regression	Sig.	Exp(B)	95.5% CI for Exp	
			Lower	Upper
Gender	0.09	0.5	0.22	1.11
Age	0.27	1.04	0.97	1.10
Duration of heroin use disorder	0.005**	1.12	1.03	1.21
IgA	0.47	1.13	0.81	1.58
IgG	0.002**	1.25	1.09	1.43
IgM	0.005**	1.80	1.19	2.71
C3	0.14	2.91	0.71	11.84
C4	0.21	0.44	0.12	1.59
RF	0.033*	1.09	1.01	1.19
Cryoglobulins	0.001**	3.57	1.72	7.41
Multivariate logistic regression				
IgM	0.026*	1.73	1.07	2.8
IgG	0.027*	1.18	1.02	1.36
cryoglobulins	<0.0001	4.43	1.99	9.83
Constant	0.001	0.05		

IgA-immunoglobulin A, IgG-immunoglobulin G, IgM-immunoglobulin M, C3-complement component 3, C4-complement component 4, RF-rheumatoid factor, Sig- Significance, Exp(B)-OR(Odds Ratio), 95.5% CI for Exp-Confidence interval

bulins (<0.001) (Table 4)

Arthralgia occurred in 59 of the 140 participants (42.14%) and was primarily located at the interphalangeal and metacarpophalangeal joints. The results of multivariate logistic regression analysis for the determination of significant factors and their predictive impact on arthralgia showed that only cryoglobulinaemia was significant. Odds ratio (OR) analysis showed that patients with cryoglobulinaemia had a 2.44-fold greater likelihood of developing arthralgia. There was no statistical significance regarding the route of heroin administration.

Skin changes were observed in 17 of the 140 participants (12.14%); they were localized in the lower extremities. The most common change was purpura in 15 of the 17 (88.23%) patients. One patient had ulcer changes, and one patient experienced erythema. These changes were more common among patients who used heroin intravenously n=13. The results of multivariate logistic regression analysis showed that only cryoglobulinaemia was a significant factor in this clinical manifestation. Odds ratio (OR) analysis showed that patients with cryoglobulinaemia had a 9.27-fold greater likelihood of developing skin changes.

Respiratory difficulties such as shortness of breath and cough were reported in 21 of the 140 participants (15.0%). Chest X-ray, blood gas analysis and spirometry were performed in all patients with respiratory difficulties. Seventeen patients had positive lung findings. Chest X-ray revealed interstitial infiltration in eight patients. Blood gas analysis indicated that three patients were hypoxemic with low partial pressure of oxygen (PaO2<10kPa), and low oxygen saturation (SaO2<90%). Spirometry revealed obstructive lung involvement in five patients and mild restrictive lung involvement in six patients with total lung capacity (TLC 80%). Respiratory difficulties were more common among patients who used heroin intravenously n=17. The results of multivariate logistic regression analysis showed that only cryoglobulinaemia was a significant factor for this clinical manifestation. Odds ratio (OR) analysis showed that patients with cryoglobulinemia had 5.29-fold greater likelihood of developing respiratory difficulties.

Neurological disorders, paraesthesia and decreased sensation in the lower extremities were reported in 18 of 140 participants (12.86%). Electromyography (EMG) was recommended for all patients but accepted by only nine. Six demonstrated an abnormal electromyography (EMG) finding (five with distal symmetric sensor neuropathy and one with sensory-motor neuropathy in the lower limbs). Neurological disorders were more common among patients who used heroin intravenously (n=14). The results of multivariate logistic regression analysis showed that only cryoglobulinaemia was a significant factor for these clinical manifestations. Odds ratio (OR) analysis showed that patients with cryoglobulinemia had 10.07-fold greater likelihood of developing neuro-

logical disorders.

Raynaud's phenomenon was reported in 27 of 140 (19.28%) patients. Only five patients underwent capillaroscopy, which was compatible with the diagnosis. Raynaud's phenomenon was more common among patients who used heroin intravenously (n=22). The results of multivariate logistic regression analysis showed that only cryoglobulinaemia was a significant factor in this clinical manifestation. Odds ratio (OR) analysis showed that patients with cryoglobulinaemia had an 11.65-fold greater likelihood of developing Raynaud's phenomenon.

Proteinuria was reported in sixty patients with an average value of 0.28±0.15g/l. There was no statistical significance regarding the route of heroin administration. The results of multivariate logistic regression analysis showed that only CIC was a significant factor for this clinical manifestation. Odds ratio (OR) analysis showed that patients with a higher level of CIC had a 3.12-fold greater likelihood of developing proteinuria.

4. Discussion

This study confirmed that patients with heroin use disorder who have more clinical manifestations also have increased mean values of the IgG fraction, the C3 component and CIC. They also have increased values above the reference ones for the IgA fraction, CIC and cryoglobulins. The onset of clinical manifestations in patients who take heroin intravenously have potential correlation with the duration of heroin use disorder, IgG fraction, IgM fraction, RF and cryoglobulins. This study showed that heroin users with cryoglobulinemia have greater likelihood of developing arthralgia, skin changes, respiratory difficulties, neurological disorders, Raynaud's phenomenon. While heroin users with a higher level of CIC have a greater likelihood of developing proteinuria.

According to the World Health Organization (WHO), the estimated use of illicit drugs is the eighth most frequent cause of disease, death, and disability in developed regions of the world. Patients with opioid use disorder suffer increased physical and mental health conditions, which are related to the adverse, toxic effects of the opioids themselves, the preparation of drugs and the route of administration, and also to the general problematic life conditions often associated with chronic opioid use [11]. An awareness of illness often appears to be missing in heroin users [5].

Combined clinical, biochemical and immunological studies have revealed certain differences among heroin users according to the route of heroin administration. Patients who primarily administered heroin intravenously had a significantly higher incidence (24%), as well as higher levels, of CIC, than the group which never administered heroin intravenously. This may be due to the more direct route and heavier antigenic challenge of the immune system by the intravenous injection of heroin, or, possibly, to additional impurities in the heroin. CICs have been similarly described in 40% of intravenous drug users (IDUs). Our study showed significantly increased values above reference levels (p=0.014) and increased mean values for CIC (p=0.003) in the symptomatic group compared with the asymptomatic group of patients with heroin use disorder. The hypocomplementemia may also be due to decreased synthesis of complement components or to increased complement catabolism as a result of heroin administration. Intravenous drug users (IDUs) who had a well-established history of nephritis within two years prior to the study were found to have abnormal urinalysis, hypocomplementaemia, RF, cryoglobulins and CIC. The clinical significance of CIC and hypocomplementaemia remains unclear in most heroin users, although they may contribute to the immunopathogenesis of nephritis in some patients [9]. Our study showed that there is a potentially significant correlation between intravenous heroin usage and duration of heroin use disorder (0.005), IgG fraction (0.002), IgM fraction (0.005), RF (0.033), and cryoglobulins (0.001).

Guo and colleagues found that heroin (diacetyl morphine) abuse could lead to an increase in the C3 and C4 complement components [4]. Our study showed significantly increased mean values of C3 component (p=0.001) in the symptomatic group compared to the asymptomatic group. Radenkova-Saeva and colleagues showed that heroin abuse lowers the level of serum C4 [8]. C3-like protein is a component of the complement system that plays a role in the development of inflammation, and could be activated by the immune response to heroin. Additional investigations are necessary on the immune response in patients with heroin use disorder following heroin administration [4].

Intravenous heroin users present more changes in certain parameters of humoral immunity, including increased levels of the IgG and IgM fractions, decreased levels of complement component C4, increased RF, anti-beta 2 glycoprotein 1 (anti-β2GP1) with the predominant IgG fraction, and cryoglobulins. Clinical manifestations are more common in cryoglobulin-positive than in cryoglobulin-negative her-

oin users [10]. In one nationwide survey in patients with cryoglobulinemia, without hepatitis C virus, the most frequent manifestations were purpura (78%), glomerulonephritis (28%), arthralgia (28%), peripheral neuropathy (22%), skin necrosis (22%), cutaneous ulcers (17%), and myalgia (11%) [12]. Our study showed that cryolobulinemia was a significant factor with predicative impact on arthralgia, skin changes, respiratory difficulties, neurological disorders and raynaud phenomenon.

Proteinuria was reported in sixty patients with an average value of 0.28±0.15g/l. There was no statistical significance regarding the route of heroin administration. The results of multivariate logistic regression analysis showed that only CIC was a significant factor in this clinical manifestation. OR analysis showed that patients with a higher level of CIC had a 3.12-fold greater likelihood of developing proteinuria.

The extent of humoral dysregulation among intravenous drug users (IDUs) had never been previously well understood. In addition to potentially contributing to inflammation-mediated co-morbidities, the humoral alterations described by Piepenbrink may have significant consequences for the protection of IDUs from pathogens, including their ability to generate effective responses to vaccines. Investigations into the impact of anti-inflammatory interventions in IDUs are warranted, and may yield wide-ranging health benefits for this growing population [6].

Limitations

This study has several limitations. It only included individuals who came to the clinic; as a result, the generalizability of the results may be limited. The biological material for the investigations was collected when the patients came to the clinic for the first time, when the most frequent clinical manifestations were observed in this study. Patients were not followed throughout the entire period of heroin use disorder.

5. Conclusions

Heroin users with cryoglobulinemia had a greater likelihood of developing arthralgia, skin changes, respiratory involvements, neurological disorders, and Raynaud's phenomenon. Some of these symptoms were more common among individuals who administered heroin intravenously. Heroin users with higher levels of CIC had a greater likelihood of developing proteinuria.

Future studies should examine immune parameters and their influence on clinical manifestations in heroin users in comparison with a healthy population. Studies should also examine the influence of immune parameters on clinical manifestations in subjects who begin to use heroin, to follow them prospectively with exposure to and abstinence from heroin, and when they are on replacement treatments. Also, considering that our findings were related to the presence of cryoglobulinaemia in these patients, but unrelated to hepatitis C infection, there is a need for further investigations on patients with heroin use disorder, to evaluate heroin (opioids) as a non-infectious cause of cryoglobulinaemia.

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Contributors

N.S., designed the study and wrote the protocol. N.S., managed the literature searches and analyses. B.Z.I., undertook the statistical analysis, and all the authors discussed the results. N.S., B.Z.I., wrote the first draft of the manuscript. The authors revised the last draft. All the authors contributed to, and have approved, the final manuscript.

Conflict of interest

The authors have no conflict of interest.

Ethics

Authors confirm that the submitted study was conducted according to the WMA Declaration of Helsinki - Ethical Principles for Medical Research Involving Human Subjects. This study was carried out with the approval of the Ethics Committee of the Medical Faculty, Skopje (date: 26.06.2008; 03-2046).

Note

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