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DRUG ADDICTION AND CHOICE OF DRUGS: TEMPERAMENT AND PERSONALITY AS RISK FACTORS

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ABSTRACT

Aims: *We sought to evaluate the risk factors and personality traits associated with specific drug use and drug addiction in general.* ***Design:*** *We compared the temperament and character traits of people addicted to opiates or alcohol to healthy controls.* ***Participants:*** *In total, 412 consecutive patients (312 people addicted opiates; 100 to alcohol) treated at the Specialised Clinic for Chemical Dependency in Belgrade, Serbia and a community sample of 346 healthy controls participated in this study.* ***Measurements:*** *We employed the Temperament and Character Inventory (TCI) and the DSM-IV criteria for opiate addiction and alcoholism.* ***Findings:*** *Participants addicted to opiates manifested "antisocial" temperaments (i.e., high novelty seeking and low reward dependence), whereas participants addicted to alcohol had "sensitive" temperaments (i.e., high novelty seeking, high harm avoidance). We observed immature personalities and personality disorders far more frequently in people addicted to opiates than those addicted to alcohol or healthy participants.* ***Conclusions:*** *Novelty seeking appears to be a risk factor for drug addiction. High harm avoidance may direct high novelty seeking people toward alcoholism. Personality disorders increase the risk of illegal drug use. Personality factors may provide useful indicators for drug addiction preventive work with young people.*

Keywords: *Temperament, Character, Drug Addiction*

INTRODUCTION

The personalities of people addicted to drugs are studied to determine their role as risk factors for drug addiction and, ultimately, to improve drug prevention and patient treatment. While most authors agree that personality traits differ between non-users and people who use drugs, only a few personality patterns have been able to predict drug addiction. One of the most consistent findings is that people who are impulsive, exploratory, and disinhibited are at higher risk for drug addiction. Specifically, novelty (1) or sensation seeking (2) are significantly higher in drug users compared to non-users (3, 4, 5). Most authors agree that these traits appear to be a risk factor for drug addiction (8).

In this article, we analyse the personality features of people addicted to opiates or those addicted to alcohol. We expected our study design to facilitate the evaluation of personality risk factors for specific types of drug use and drug addiction in general. We chose opiates and alcohol because opiates are illegal, and their abuse may reflect immature personalities associated with law breaking, whereas alcohol is anxiolytic and its abuse may be associated with people who feel the need to self-medicate their stress and high anxiety.

We are aware of only one prior study in which the Temperament and Character Inventory (TCI; 1) assessed personality features of people addicted to opiates, those addicted to alcohol, and healthy participants in Liege, Belgium (6). These authors found robust differences in temperament and personality among the three groups. Our study attempted to replicate these findings using the same personality test but in a different culture (East vs. West), socio-economic setting (developing vs. developed countries), and level of social stability (society in transition in Belgrade vs. stable society in Belgium).

One unique feature of our study is the permissive environment in Belgrade, Serbia that provides easy access to street drugs (although heroin is illegal in Belgrade, as it is most everywhere in the world, unstable social circumstances interfere with the effective policing of illegal drug trafficking). Such an environment is optimal to investigate whether one's drug choice is based on one's preference or its availability (i.e., do addicts use what they like or what is available?).

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METHODS

a) Sample

We conducted this study in the Specialised Clinic for Chemical Dependency, Belgrade, Serbia. The participants were consecutive patients admitted to the clinic for residential detoxification and rehabilitation.

Table 1. People addicted to opiates, people addicted to alcohol, and healthy controls. Gender distribution and sample size

	People addicted to opiates	People addicted to alcohol	Healthy controls	Total
Female	66	36	177	279
Male	246	64	169	479
Total	312	100	346	758

Table 1 divides all participants by their gender and addiction. We recruited the control group from the local School of Nursing at the University of Belgrade and the Belgrade Police Academy.

Table 2. Age distribution

	People addicted to opiates	People addicted to alcohol	Healthy controls
Female	26.4 (6.22)	36.8 (11.88)	24.6 (7.85)
Male	26.3 (5.9)	40.5 (10.5)	21.9 (5.14)
Male and female	26.32 * (5.99)	39.21** (11.1)	23.33*** (6.79)

* sig different from people addicted to alcohol (<.0001) and controls (<.05)
** sig different from people addicted to opiates (<.0001) and controls (<.0001)
*** sig different from people addicted to opiates (<.05) and people addicted to alcohol (<.0001)

Table 2 shows the average age for all groups of participants. Participants addicted to alcohol were significantly older than the other two groups (p <.0001).

b) Diagnostics

A psychiatrist (BL, PN) diagnosed both alcohol and opiate dependency using the DSM-IV criteria.

Exclusion criteria:

Participants who were addicted to both alcohol and opiates (or to other classes of drugs) were not included in this study.

Participants with comorbid Axis I disorders were not included in the study.

c) Personality Measures

The TCI (1) is a self-report true-false test consisting of 239 items designed to measure four temperament traits (i.e., harm avoidance, novelty seeking, reward dependence, and persistence) and three character traits (i.e., self-directedness, cooperativeness, and self-transcendence). Each of these dimensions of temperament and character is subdivided into five lower-order traits (or facets). The TCI is psychometrically valid in both the US (7) and in Serbia (8).

d) Statistical analyses

The variables available for analysis were limited to age, gender, the TCI personality dimensions and substance abuse group (i.e., opiate use, alcohol use, or control). Because age and gender were distributed unevenly in the substance abuse categories and because there are known, albeit slight, age and gender effects associated with TCI scores for NS, HA, RD and CO, we opted to test the hypothesis that group membership is associated with personality by conducting three separate logistic regressions, each including two of the three groups.

In each of the pair-wise logistic regressions, we entered age and gender as covariates along with the standardised scores (mean = 0, SD = 1) for the seven personality dimensions of the TCI. Group membership was the dependent variable.

In earlier work (9), we demonstrated that TCI personality scores could efficiently diagnose the probability of a personality disorder. To examine differences in character maturity in this study, we categorised participants into three groups: 1) "probable personality disorder" (the bottom 1/6th of the character traits scores); 2) "immature personalities" (the next 1/6th of the character scores); and 3) "mature personalities" (the top 2/3rd) based on the distribution of maturity in healthy controls. We then reviewed the distribution of these types within the three groups.

We conducted all analyses using SAS version 9.1 (10).

RESULTS

As shown in Tables 3, 4, 5, and 6, pair-wise logistic regressions comparing the groups revealed a number of significant relationships between personality traits and drug addiction.

People addicted to opiates versus healthy controls

With respect to temperament traits (Tables 3 and 6), people addicted to opiates were significantly higher in novelty seeking and significantly lower in reward dependence compared to healthy controls. This temperament configuration is called "antisocial" because these people manifest impulsive behaviours with little regard or empathy for others. Furthermore, people addicted to opiates were significantly lower in self-directedness and higher in self-transcendence than healthy participants. These character scores reflect participants' greater likelihood to be immature and have a personality disorder (low self-directedness) as well as to be susceptible to day dreaming, fantasy, and magical thinking (high self-transcendence).

People addicted to alcohol versus healthy controls

With respect to temperament traits (Tables 4 and 6), people addicted to alcohol were significantly higher in novelty seeking and harm avoidance compared to healthy controls. This temperament configuration is called "sen-

Table 3. LOGISTIC REGRESSION MODEL: Predicting group membership from personality dimensions
Opiates and Healthy controls

Parameter	DF	Estimate	Standard error	Wald's Chi -square	Pr > Chi square
Intercept	1	- 1.2713	0.4942	6.6175	.0101
Age	1	0.1497	0.0186	64.5351	.0001
Sex	1	- 1.9706	0.2485	62.8972	.0001
Novelty Seeking	**1**	**1.2838**	**0.1516**	**71.7408**	**.0001**
Harm Avoidance	1	0.1142	0.1360	0.7049	.4011
Reward Dependence	**1**	**- 0.2786**	**0.1209**	**5.3146**	**.0211**
Persistence	1	0.1064	0.1147	0.8610	.3535
Self-Directedness	**1**	**- 0.4504**	**0.1637**	**7.5718**	**.0059**
Cooperativeness	1	- 0.1332	0.1381	0.9306	.3344
Self-Transcendence	**1**	**0.3093**	**0.1168**	**7.0063**	**.0081**

Significant differences are presented in bold

Table 4. LOGISTIC REGRESSION MODEL: Predicting group membership from personality dimensions
People addicted to alcohol and healthy controls

Parameter	DF	Estimate	Standard error	Wald's Chi -square	Pr > Chi square
Intercept	1	- 5.6408	0.7287	59.9297	.0001
Age	1	0.1970	0.0201	95.8615	.0001
Sex	1	- 0.8960	0.3545	6.3890	.0115
Novelty Seeking	**1**	**0.5432**	**0.2021**	**7.2246**	**.0072**
Harm Avoidance	**1**	**0.4450**	**0.2091**	**4.5266**	**.0334**
Reward Dependence	1	0.0079	0.1905	0.0017	.9666
Persistence	1	0.2327	0.1932	1.4509	.2284
Self-Directedness	1	- 0.3152	0.2612	1.4555	.2277
Cooperativeness	1	- 0.2823	0.2418	01.3633	.2430
Self-Transcendence	**1**	**- 0.3700**	**0.1908**	**3.7616**	**.0524**

Significant differences are presented in bold

Table 5. LOGISTIC REGRESSION MODEL: Predicting group membership from personality dimensions
People addicted to alcohol and people addicted to opiates

Parameter	DF	Estimate	Standard error	Wald's Chi -square	Pr > Chi square
Intercept	1	- 7.0316	0.8523	68.0691	.0001
Age	1	0.1440	0.0193	55.4923	.0001
Sex	1	1.1058	0.3550	9.7036	.0118
Novelty Seeking	**1**	**- 0.5413**	**0.1989**	**7.4092**	**.0065**
Harm Avoidance	1	0.1537	0.1814	0.7176	.3969
Reward Dependence	1	0.1430	0.1847	0.5994	.4388
Persistence	1	0.1565	0.1641	0.9102	.3401
Self-Directedness	1	- 0.1180	0.2377	0.2463	.6197
Cooperativeness	1	0.2084	0.2144	0.9448	.3310
Self-Transcendence	**1**	**- 0.5900**	**0.1645**	**12.8697**	**.0003**

Significant differences are presented in bold

Table 6. Pair-wise comparison between groups. Odds ratio estimates, 95% Wald Confidence limits, p-values			
	Normal/Alcohol Point estimate (confidence limits), p	**Normal/Opiate** Point estimate (confidence limits), p	**Alcohol/Opiate** Point estimate (confidence limits), p
Age	**1.21 (1.17-1.27)** *	**1.16 (1.12-1.20)** *	**1.15 (1.11-1.20)** *
Sex	**0.40 (0.20-0.82)** ◊	**0.14 (0.09-0.23)** *	**3.02 (1.50-6.06)** ***
NS	**1.72 (1.16-2.56)** ***	**3.61 (2.68-4.86)** *	**0.58 (0.39-0.86)** ***
HA	**1.56 (1.04-2.35)** ◊	1.12 (0.86-1.46)	1.17 (0.82-1.66)
RD	1.01 (0.69-1.46)	**0.76 (0.60-0.96)** **	1.15 (0.80-1.66)
PER	1.26 (0.86-1.84)	1.11 (0.89-1.39)	1.17 (0.85-1.61)
SD	0.73 (0.44-1.22)	**0.64 (0.46-0.88)** ***	0.89 (0.56-1.42)
CO	0.75 (0.47-1.21)	0.88 (0.67-1.15)	1.23 (0.81-1.87)
ST	0.69 (0.47-1.00)	**1.37 (1.08-1.71)** ***	**0.55 (0.40-0.76)** **

p-values
* (<.0001), ** (<.001), *** (<.01) , ◊ (< .05)

sitive" because these people frequently manifest coexisting anxiety and impulsivity (i.e., they perceive novelty as simultaneously appealing and potentially harmful). With respect to character traits, people addicted to alcohol had significantly lower self-transcendence compared to healthy controls.

People addicted to alcohol versus people addicted to opiates

The two groups of addicts differed significantly in novelty seeking and self-transcendence; both traits were higher in people addicted to opiates (Tables 5 and 6). In addition to being a risk factor for drug addiction in general, high novelty seeking apparently increases the specific risk of opiate addiction compared to alcoholism. This finding is especially true when coupled with high self-transcendence, which as noted above, makes people susceptible to fantasy and day dreaming (features potentiated by opiates).

Character Scores

As shown in Table 7, the frequencies of immature and mature personalities in the opiate group were significantly higher compared to a healthy sample. (The prevalence of personality disorders in the general population is estimated to be around 15%). We classified 67% of healthy controls and 64% of people addicted to alcohol as having mature personalities. Conversely, only 33.3% of people addicted to opiates had mature personalities; 66.7% were classified as having either a probable personality disorder or an immature personality. These differences were statistically significant at p <.0001.

Table 7. Groups by character maturity (probable PD → Mature Character)				
Number Percent Row Pct Col Pct	Prob PD (Bottom 1/6)	Immature (Next 1/6)	Top 2/3 (Mature)	Total
Normal	54 7.12 15.61 25.00	60 7.92 17.34 **37.07**	**232** **30.61** **67.05** **48.33**	346 45.65
Alcohol	18 2.37 18.00 8.61	18 2.37 18.00 12.08	**64** **8.44** **64.00** **16.00**	100 13.19
Opiate	**137** **18.07** **43.91** **65.55**	**71** **9.37** **22.76** **47.65**	104 13.72 33.33 26.00	346 45.65
Total	209 27.57	149 19.66	400 52.77	758 100

DISCUSSION

Initial exposure to and the repetitive use of drugs are the initial two steps in the aetiopathogenesis of drug addiction. Personality factors seem to influence each of these steps.

Our results demonstrate that people addicted to opiates and those addicted to alcohol show higher novelty seeking than healthy controls. This finding is in accord with prior studies in different cultures (3, 6); in other words, novelty seeking may be a risk factor for drug addiction because it increases the likelihood of initial drug experimentation.

After initial experimentation, the repetitive use of drugs triggers a series of structural central nervous system adaptations that leads to compulsive drug seeking and full-blown drug addiction. Our study indicates that both personality factors and the psychological effect of the drug may perpetuate repetitive drug use.

As shown in Tables 5 and 6, people addicted to opiates showed higher novelty seeking and higher self-transcendence compared to those addicted to alcohol. These traits make people easily bored, exploratory, prone to magical thinking and daydreams, and increase the need for frequent stimulation (1). Thus, high novelty seeking coupled with high self-transcendence and frequent boredom lead people to use opiates, whose euphoric rush maximises positive moods and facilitates magical thinking. Indeed, most our participants addicted to opiates reported that they chose to use opiates to "get high and feel good".

On the other hand, participants with alcohol dependence (Tables 4, 6) showed high novelty seeking and high harm avoidance, the latter of which reflects a susceptibility to stress and anxiety (also called "trait anxiety"). Chronic vulnerability to stress (harm avoidance) may have led to alcohol abuse to alleviate negative moods or reduce anxiety. Indeed, most of our participants who were addicted to alcohol reported that their motivation to drink is to "get relief" and "forget their worries". Thus, high harm avoidance may direct people high in novelty seeking toward alcoholism. Of note, Cloninger et al. (11) found that harm avoidance inhibits the initiation and frequency of drinking but increases the risk of developing an addiction once frequent drinking has begun.

In addition to temperamental and personality traits, personal maturity and social attachment appears to influence one's drug of choice. Specifically, people addicted to opiates showed lower reward dependence than people addicted to alcohol or healthy controls. Low reward dependence is common in people who are socially detached, aloof, and insensitive to social approval. These traits make choosing illegal drugs or illegal means to obtain drugs easier. Likewise, one of the most striking findings in our study was the disproportionate frequency of immature personalities and personality disorders found in people addicted to opiates compared to those addicted to alcohol and healthy controls (Table 7). We classified a significantly higher percentage of healthy participants and participants addicted to alcohol as having mature personalities compared to those addicted to opiates. Conversely, we classified a significantly higher percentage of people addicted to opiates as having a probable personality disorder or an immature personality. Taken together with low reward dependence, personality disorders may explain illegal (e.g., opiates) versus legal drug use (e.g., alcohol). People who are addicted to alcohol, however, tend to score higher for reward dependence and to have mature personalities (only 18% had probable personality disorders in our sample). This trait reduces the risk of illegal drug use and increases the likelihood of choosing legally obtained substances.

Of note, LeBon (6) found that people addicted to alcohol tended to have more immature personalities compared to people addicted to opiates, indicating that drug availability may influence drug choice more than personal maturity in non-permissive environments.

Drug choice in permissive versus non-permissive environments

Personality factors can differentiate addicts in the same addiction class from each other. Based on family and personality studies, Cloninger et al. (5) described two types of alcoholism: Type I is characterised by late onset, anxious temperaments (i.e., high harm avoidance) and minimal criminality. Conversely, Type II is characterised by early onset, high impulsivity (i.e., high novelty seeking), and serious criminality.

Note that in our study, people addicted to opiates differed from those addicted to alcohol in the way that Cloninger's Type I and II diverge. People addicted to opiates were younger and showed high novelty seeking and antisocial traits, whereas those addicted to alcohol were older and showed high harm avoidance and fearful traits. Type I and Type II alcoholism classification was determined in the U.S. and Sweden—non-permissive societies with strict laws against illegal drug use. The environment in Belgrade, on the other hand, is permissive and provides easy access to street drugs. Thus, in permissive environments, young, exploratory, and antisocial drug users choose opiates over alcohol, whereas older, anxious people prefer alcohol. The type I and II alcoholism classifications appear to at least partly reflect the availability and accessibility of drugs in non-permissive societies (i.e., if given a choice, young antisocial addicts will use opiates).

Figure 1 summarises the personality characteristics of people addicted to opiates and those addicted to alcohol.

CONCLUSION

Together with LeBon et al. (6), the results presented in this paper outline a consistent and predictable relationship between personality features and drug addiction. These results can help researchers design efficient prevention strategies and specific treatments for people addicted to drugs. We are delighted to report that we have proposed screening 6th, 7th, and 8th graders at high risk (those high in Novelty Seeking) for drug use in Belgrade. Moreover, we use preventive and intensive education as well as occupational interventions with high-risk youths and their families to minimise the chance of becoming addicted to drugs.

Our study is limited because it is retrospective (i.e., the personality factors are studied in people addicted to drugs; thus, we cannot assess the causality our findings). Causal hypotheses are more realistic if prospective follow-up studies are able to replicate our results.

On the positive side, our control group was younger than either addiction group, which adds weight to the finding that novelty seeking is a risk factor for drug addiction. (We expected healthy controls would show high novelty seeking because young people are typically novelty seekers).

Figure 1. Summary of personality differences between people addicted to opiates and those addicted to alcohol

	Alcohol	Opiates
Novelty Seeking	↑↑	↑↑
Harm Avoidance	↑	Ø
Personality Disorder	Ø	↑↑
Self-Transcendence	↓↓	↑↑
Age	Older (I)	Younger (II)

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INFLUENCE OF MODULATORS OF RELAXANT EFFECT OF PENTOXYPHYLLINE IN ISOLATED RAT UTERUS

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UTICAJ MODULATORA RELAKSANTNOG EFEKTA PENTOKSIFILINA NA IZOLOVANOM UTERUSU PACOVA

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ABSTRACT

Background. *Pentoxyphylline is a methylxanthine derivative used in the treatment of peripheral vascular diseases. One effect of pentoxyphylline action is the vasodilatation of blood vessels. In this study, the effect of increasing concentrations of pentoxyphylline on contractility of isolated rat uteri was examined.*

Methods. *Uteri were isolated from virgin Wistar rats (180–220 g) and suspended in an isolated organ bath chamber containing De Jalon's solution and aerated with 95% O2 and 5% CO2. The temperature was maintained at 37°C. Isometric contractions were recorded using an isometric force transducer (Ugo Basile). The preload of the preparation was about 1 g. Uteri were allowed to contract spontaneously or in the presence of Ca2+ (6 mM) and were treated with pentoxyphylline.*

Results. *Pentoxyphylline caused concentration-dependent inhibition of spontaneous rhythmic uterine activity and uterine activity induced by calcium. We showed that the inhibitory effect of pentoxyphylline depends on the type of muscle contraction activation, and that it is significantly stronger in spontaneous contractions induced by calcium Ca2+. As opposed to methylene blue, L-arginine and glibenclamide did not antagonise the relaxing effect of pentoxyphylline on the isolated rat uterus.*

Conclusion. *Our results suggest that the signaling pathway by which pentoxyphylline causes relaxation of uterine muscle cells does not involve NO because the presence of L-arginine did not affect the action of the drug; however, it may depend on an NO-independent cGMP signaling pathway because the presence of methylene blue significantly antagonised the effect of pentoxyphylline. These results indicate that pentoxyphylline could be a potential tocolytic drug.*

Key words: *pentoxyphylline, rat uterus, L-arginine, glibenclamide, methylene blue*

Running title: *Pentoxyphylline Inhibits Contractility of Rat Uterus*

SAŽETAK

Cilj. *Pentoksifilin, koji se koristi za lečenje perifernih vaskularnih obolenja, je derivat metilksantina. Jedan od načina delovanja pentoksifilina je prouzrokovanje vazodilatacije krvnih sudova. U ovom radu ispitivali smo efekt rastućih koncentracija pentoksifilina na kontraktilnost izolovanog uterusa pacova.*

Metode. *Uterusi, koji su izolovani od neparenih ženki pacova Wistar soja (180-220 g),držani su u kupatilu za izolovane organe na temperaturi od 37°C, u De Jalon-ovom rastvoru kroz koji je propuštana mešavina od 95% kiseonika i 5% ugljendioksida. Izometrijske kontrakcije su registrovane korišćenjem izometrijskog transducera Ugo Basile, pri opterećenju preparata od 1 g. Efekt pentoksifilina je ispitivan na kontrakcije za vreme spontane ritmičke aktivnosti i u prisustvu kalcijuma (6 mM),*

Rezultati. *Pentoksifilin je prouzrokovao koncentracijski-zavisnu inhibciju spontane rithmičke aktivnosti, kao i fazne aktivnosti prouzrokovane kalcijumom. Inhibitorni efekt pentoksifilina zavisio je od tipa aktivacije glatkog mišića uterusa. On je ispoljio značajno jači relaksantni efekt na kontrakcije prouzrokovane kalcijumom. Nasuprot metilenskom plavilu, L-arginin i glibenklamid ne antagonizuju relaksantni efekt pentoksifilina na izolovanom uterusu pacova.*

Zaključak. *Dobijenii rezultati sugerišu da signalini putevi sa kojima pentoksifilin prouzrokuje relaksaciju glatkih mišičnih ćelija uterusa, za razliku izolovanih krvnih sudova, verovatno ne uključuje u većoj meri prisustvo NO (jer prisustvo L-arginine nije menjalo efekt ovog leka), ali zavisi od cGMP signalinih puteva nezavisnih od NO (zbog toga što prisustvo metilenskog plavila značajno antagonizuje njegov efekt). Ovi rezultatia ukazuju da bi pentoksifilin mogao da bude potencijalni tokolitički lek.*

Ključne reči: *pentoksifilin, uterus pacova, L-arginin glibenclamid i metilensko plavilo*

Kratki naslov: *pentoksifilin inhibiše kontrakcije uterusa pacova*

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INTRODUCTION

Tocolytics, such as β2-adrenergic agonists, are frequently used to prevent miscarriage and premature birth, however these compounds are associated with insufficient efficacy and excessive side-effects.[1] Accordingly, there is a need to identify novel drugs with tocolytic characteristics, such as calcium agonists, potassium channel openers and other vasodilators.[2] It has been shown that even otomolar concentrations of nicardipine inhibit spontaneous rhythmical activity of the isolated uterus.[3] Nitric oxide (NO) is involved in numerous physiological processes and pathological conditions, and it mediates the relaxing effect of protamine sulphate and other smooth muscle vasodilators.[4] In cells, NO is created under the influence of NO-synthesis.[5] High doses of L-arginine increase blood flow in the heart, mesenterium, lungs and liver without affecting total peripheral resistance and blood pressure.[6] L-arginine, however, can cause significant hypotension in normotensive rats pretreated with physostigmin.[7] Previous reports showed increased NO synthesis during normal pregnancy in animals. In humans, lack of NO causes vasoconstriction and pre-eclampsia. NO is characterised by extreme reactivity to intracellular enzymes and has been shown to affect activity of guanylate cyclase (GC). Reaction between NO and GC can be inhibited with methylene blue.[8]

Pentoxyphylline is a methyl xanthine derivative used to treat peripheral vascular diseases. Potential indications for this drug, as well as its mechanism of action at the molecular level, are being intensively studied.[9]

In previous studies, we showed that the endothelium plays a significant role in the relaxing mechanism of pentoxyphylline in isolated rat mesenteric arteries. In the present study, we examined the effects of increasing pentoxyphylline concentrations on spontaneous rhythmical activity of isolated uteri and on calcium chloride-caused activity in the uterus. To elucidate the mechanism of action of pentoxyphylline in uterine smooth muscle, we studied the drug's effects in the presence of L-arginine (an NO precursor), glibenclamide (a potassium channel antagonist) and methylene blue (a GC inhibitor).

MATERIALS AND METHODS

All protocols for handling rats were approved by the local Ethical Committee for Animal Experiments, which strictly follows international regulations. Isolated uteri of virgin Wistar rats (200–250 g) in oestrus, as determined by daily examination of vaginal lavage, were used in this study. Each uterus was suspended in an isolated organ bath chamber (Ugo Basile) containing De Jalon's solution (NaCl 9.0 g/l, KCl 0.42 g/l, $NaHCO_3$ 0.5 g/l, $CaCl_2$ 0.06 g/l, glucose 0.5 g/l) and aerated with 95% O_2 and 5% CO_2. The temperature was maintained at 37°C. Isometric contractions were recorded using an isometric force transducer (Ugo Basile). The uteri, which were either spontaneously active or induced with 6 mM Ca^{2+}, were allowed to equilibrate at 1 g of tension before experimental drugs were added. After establishing stable spontaneous contractions (approx. 20 min), uteri were treated with increasing concentrations of pentoxyphylline (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 mM and 192.3 mM) until total cessation of contractions. To explore the mechanism of action of pentoxyphylline on the uterus smooth muscle, we studied its effects in the presence of L-arginine (0.3 µmol), glibenclamide (2×10^{-6} mol/l) and methylene blue (0.9×10^{-6} mol/l), which were added to De Jalon's solution 10 min before pentoxyphylline.

The effects of these treatments on uterine contractions were calculated as the percentage of control contractions in untreated uteri. All data are expressed as the mean ± SEM. Differences between groups were tested by two-way ANOVA with treatment and dose as factors. Differences between groups were considered statistically significant when $p < 0.05$.

Pentoxyphylline, methylene blue, L-arginine and glibenclamide were purchased from Sigma-Aldrich (St. Louis, MO, USA). Salts for De Jalon's solution were obtained from ZORKA Pharma (Sabac, Serbia) and Merck (Darmstadt, Germany). All drugs were dissolved in distilled water except for glibenclamide, which was dissolved in polyethylene glycol.

RESULTS

Effect of pentoxyphylline on spontaneous rhythmical activity and Ca^{2+}-induced contractions of isolated rat uterus

Increasing pentoxyphylline concentrations (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 mM and 192.3 mM) resulted in concentration-dependent inhibition of spontaneous rhythmical activity and calcium-induced contractions of the isolated rat uterus. The degree of pentoxyphylline inhibitory effect depended on activation type. Pentoxyphylline exhibited a stronger relaxing effect on calcium-induced uterus contractility. For instance, pentoxyphylline concentration of 106.9 mM, which completely inhibited calcium-induced contractions (98.08%), only partially inhibited spontaneous rhythmical activity of the isolated rat uterus (52.94%). Total inhibition of calcium-induced contractions was achieved with lower concentrations of pentoxyphylline (64.1 mM). In the case of calcium-induced contractions, pentoxyphylline caused time-dependent contraction inhibition; increasing pentoxyphylline concentrations resulted in increased duration of the calcium-induced contractility (Figure 1A, B and C).

Effect of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uterus in the presence of glibenclamide and L-arginine

In this series of experiments, we showed that the presence of glibenclamide (2×10^{-6} mol/l) stimulates the relaxant effect of increasing concentrations of pentoxyphylline on the spontaneous rhythmical activity of the isolated rat uterus. In control experiments without glibenclamide,

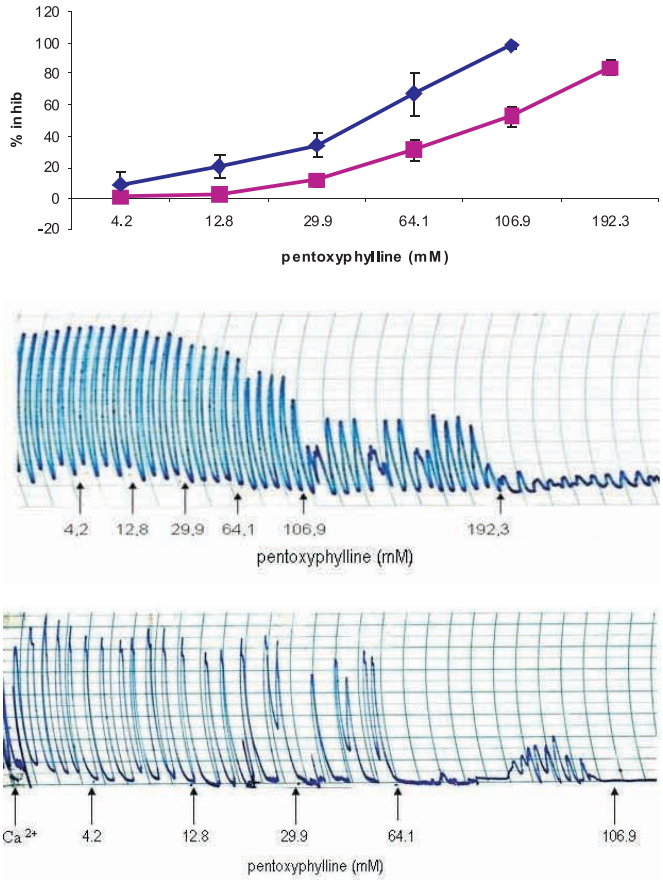


Figure 1.
(A) Concentration–response curves for pentoxyphylline relaxation on the spontaneous rhythmic contractions (closed square ■) and on the contractions provoked by Ca^{2+} (closed diamond ♦) of the isolated rat uterus. The amplitude of contractions just before addition of pentoxyphylline was taken as 100%. The data points represent mean values and the vertical lines indicate the S.E.M. (n = 8–12).
(B) A representative original trace of spontaneous uterine contractions induced by pentoxyphylline at various concentrations (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 mM and 192.3 mM).
(C) A representative original trace of Ca^{2+}-induced contractions of the rat uterus treated with pentoxyphylline (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM and 106.9 mM).

pentoxyphylline caused significant inhibition of spontaneous rhythmical activity at a concentration of 192.3 mM. On the other hand, in most experiments a pentoxyphylline concentration of 66.3 mM was sufficient for total inhibition of spontaneous rhythmical contractions in the presence of glibenclamide (Figure 2A and B).

We also studied the effects of increasing concentrations of pentoxyphylline on spontaneous rhythmical activity of isolated the rat uterus and calcium-induced contractions in the presence of L-arginine (0.3 μmol). In the presence of L-arginine, pentoxyphylline did not change its inhibitory effect on spontaneous rhythmical activity or calcium-induced activity.

Influence of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uterus in the presence of methylene blue

In these experiments we studied the effects of increasing concentrations of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uterus in the presence of methylene blue (0.9×10^{-6} mol/l). Methylene blue antagonised the relaxing effect of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uterus. For example, in the presence of methylene blue, even the highest concentration of pentoxyphylline, did not cause complete inhibition of uterine contractions (Figure 3A and B). Interestingly, the addition of pentoxyphylline inhibited uterine contractions, however this inhibition quickly disappeared, and contractions returned to control levels (Figure 3B.).

DISCUSSION

In this study, we examined the effects of increasing concentration of pentoxyphylline on the spontaneous rhythmical activity and Ca^{2+}-induced contractions of the isolated rat uterus. Pentoxyphylline induced concentration-dependent inhibition of spontaneous rhythmic uterine activity and uterine activity caused by calcium. We showed that the degree of inhibition effect by pentoxyphylline depends on the type of muscle contraction activation, and that it is sig-

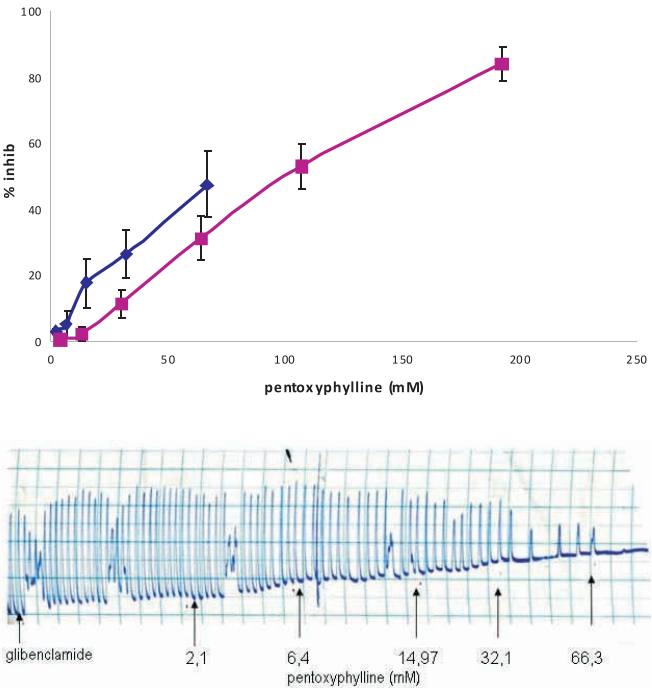
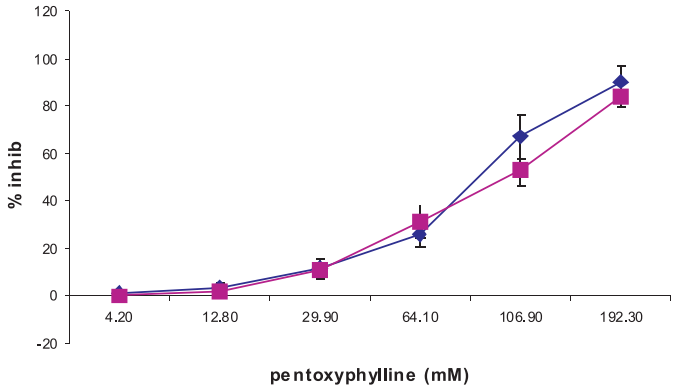


Figure 2.
(A) Effect of pentoxyphylline (4,2 mM, 12,8 mM, 29,9 mM, 64,1 mM, 106,9 mM and 192,3 mM) on the spontaneous rhythmic contractions of rat uteri in the presence (closed diamond ♦) and absence of glibenclamide (2×10^{-6} mol/l) (■ closed square). Contractile activity was expressed as the relative ratio between mean height peak of untreated control and treated uteri. Data are expressed as the mean ± s.e.mean ($n = 8^{-12}$). Pretreatment with glibenclamide significantly increased the relaxing effect of pentoxyphylline ($p < 0.0001$).
(B) A representative original trace showing the effect of (2.1 mM, 6.4 mM, 14.9 mM, 32 mM and 66,3 mM) on the spontaneous rhythmic contractions of the rat uterus, in the presence of glibenclamide.



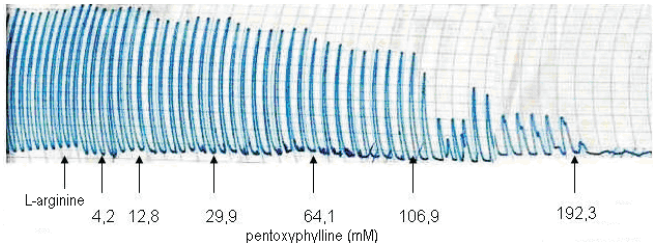


Figure 3.
Effect of pentoxyphylline (4,2 mM, 12,8 mM, 29,9 mM, 64,1 mM, 106,9 mM and 192,3 mM) on the spontaneous rhythmic contractions of rat uteri with (closed diamond ♦) or without L-arginine (2×10^{-6} mol/l) (closed square ■). Contractile activity was expressed as the relative ratio between mean height peak of untreated control and treated uteri. Data are expressed as the mean ± s.e.mean. ($n = 8^{-12}$). (B) A representative original trace showing the effect of pentoxyphylline (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 and 192.3 mM) on the spontaneous rhythmic contractions of the rat uterus in the presence of L-arginine.

nificantly stronger in spontaneous contractions caused by calcium. These results are consistent with pentoxyphylline being a possible drug that could be used for prevention of miscarriages and premature births.

Apart from finding that pentoxyphylline decreases uterine contractility in a concentration-dependent manner, we also noticed that the degree of the inhibitory effect depended on muscle contraction activation type. That is, pentoxyphylline exhibited differential effects spontaneous rhythmical and spontaneous calcium chloride-induced. The same applied concentrations of pentoxyphylline potently inhibited spontaneous rhythmical activity of the uterus. On the other hand, complete inhibition of rhythmical activity caused by calcium-chloride was achieved with lower pentoxyphylline concentrations.

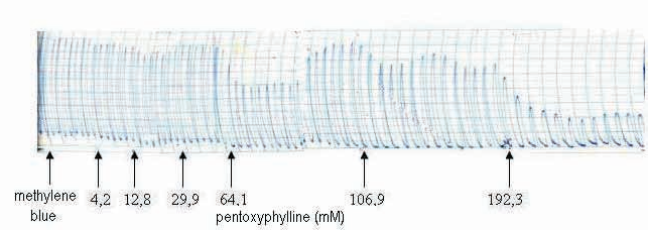


Figure 4.
A representative original trace showing the effect of pentoxyphylline (4.2 mM, 12.8 mM, 29.9 mM, 64.1 mM, 106.9 and 192.3 mM) on the spontaneous rhythmic contractions of the rat uterus in the presence of methylene blue.

Our results showing that the degree of the pentoxyphylline inhibitory effect depends on muscle activation type are consistent with previous reports showing calcium antagonism of uterine smooth muscle contraction. For example, nitrendipine potently inhibited contractions of the isolated rat uterus induced by electrical stimulation, while showing weaker inhibitory effects on spontaneous rhythmical activity and acetylcholine-induced activity, and the weakest effect was observed on oxytocin-induced activities.[3,10]

Spontaneous rhythmical muscle activity is achieved by calcium influx into cells from extracellular spaces through calcium channels located in the cellular membrane. Consequently, spontaneous rhythmical activity depends primarily on calcium concentrations outside the cell. Other types of muscle activation (e.g., induced by acetylcholine or oxytocin) depend on intracellular calcium or calcium that enters the cell by activation of muscarine and oxytocin receptors.[10]

Our results show that pentoxyphylline had a weaker relaxing effect on spontaneous rhythmical activity than on muscle activity in the presence of exogenous extracellular calcium. This result suggests that spontaneous rhythmical activity of muscles could depend on the calcium concentration present in the cell to a greater extent than previously thought.

L-arginine is a precursor of NO and, as such, it can raise the NO concentration, leading to smooth muscle cell relaxation. In human and animal models unable to produce NO due to endothelium dysfunction, L-arginine restores endothelium-dependent vasodilatation. However, in our experiments we found that L-arginine did not antagonise the relaxing effect of pentoxyphylline on the isolated rat uterus. These results suggest that pentoxyphylline achieves its relaxing effect on uterine smooth muscle regardless of the presence of nitric oxide.

In contrast with our findings, in experiments involving renal and mesenteric arteries taken from normotensive and hypertensive rats, it was found that L-arginine antagonised relaxation caused by sodium nitroprusside.[11] The authors proposed a possible explanation of this phenomenon based on the ability of sodium nitroprusside to achieve its action through peroxynitrate and not through S-nitrosothiole.

In our experiments, we found that the relaxing effect of pentoxyphylline was enhanced by the presence of glibenclamide, a selective blocker of K_{ATP} channels. Complete inhibition of contractions in the presence of glibenclamide was achieved using lower pentoxyphylline concentrations.

Potassium channels are present in numerous smooth muscles of the uterus.[12] Recent results show that some known potent vasodilators, such as sodium nitroprusside (an NO donor) and minoxidil, act in part by opening of potassium channels.[13] One of the most studied types of potassium channels, which is dominant in smooth muscles of the uterus, is a large calcium-dependent potassium channel, BK_{ca} or maxi K. This potassium channel is important during gestation and, in particular, during delivery, because its inhibition leads to increased intracellular calcium

levels necessary for birth contractions.[14] ATP-dependent potassium channels (K_{ATP}) also play an important role in uterine smooth muscle physiology; these channels form the connection between the metabolic state of the cell and cell excitation (i.e., contractility).[15]

Changes in the expression and activity of potassium channels are very important for uterus contractility control.[2] For example, H_2O_2 induces concentration-dependent relaxation of the isolated rat uterus via voltage-dependent potassium channels.[16] Based on these findings, studying potassium channel modulators and their effect on NO in uterine tissue is important to better understand uterine physiology and pathophysiology and to help identify new therapeutic concepts in the treatment of uterus contractility disturbances.[2,3] Together with detailed information about potassium channels, there is a pronounced pharmaceutical interest in the synthesis and development of selective potassium channel modulators, as well as re-evaluation of the spectrum of choice of existing drugs and substances influencing permeability of smooth muscle cells membranes for potassium ions (especially vasodilators with so-called direct effects, including monoxidil and diazoxide).

It has been shown that glibenclamide leads to inhibition of contractions of arterial muscle elements, probably by inter-reacting with voltage-dependent calcium channels and by blocking.[17] Our results could be interpreted in the same way. It is possible that glibenclamide stimulates the relaxing effect of pentoxyphylline by blocking voltage-dependent calcium channels, thereby decreasing calcium entry into the cell, causing mus

Methylene blue inhibits production of cGMP by preventing interaction of NO with guanylate cyclase. In our experiments, methylene blue decreased the relaxing effect of pentoxyphylline. Not even the highest applied concentration of pentoxyphylline achieved complete discontinuation of contractions. It is interesting that inhibition caused by pentoxyphylline in the presence of methylene blue has a short duration. That is, while pentoxyphylline inhibited uterus contractions, the duration of this inhibition was brief and contractions returned to the values of the control level.

Data showing that methylene blue antagonises the relaxing effect of sodium nitroprusside are consistent with our results.[5] Methylene blue blocks the activation of guanylate cyclases, thereby preventing muscle relaxation. cGMP activates cGMP-dependent protein kinase, which blocks the entrance of calcium ions, activates potassium channels and decreases levels of IP_3, leading to vasodilatation. However, it has also been shown that methylene blue fails to antagonise the relaxing effect of sodium azide and of nitroglycerol.[18]

Out results suggest that NO is not significantly involved in the realisation of pentoxyphylline effects. There are data about the existence of signaling pathways in the cell, including NO-independent cGMP creation, which does not lead to relaxation. It is possible that methylene blue influences these signaling pathways, leading to weaker relaxing effects of pentoxyphylline in interaction with methylene blue.

According to previous reports, pentoxyphylline acts as a phosphodiesterase blocker.[19] By blocking phosphodiesterase, pentoxyphylline directly increases cAMP levels in muscle cells, leading to muscle relaxation. Since methylene blue, a known inhibitor of guanylate cyclase, decreased the inhibitory effect of pentoxyphylline, it is possible that even cGMP is included in muscle relaxation via NO-independent signaling pathways.

CONCLUSIONS

Pentoxyphylline caused concentration-dependent inhibition of spontaneous rhythmical activity and calcium-induced contractions in isolated rat uteri. The magnitude of the relaxing effect of pentoxyphylline depended on the type of uterus smooth muscle activation. As opposed to methylene blue, L-arginine and glibenclamide did not antagonise the relaxing effect of pentoxyphylline on spontaneous rhythmical activity of the isolated rat uteri. These observations suggest a possible mechanism of action of pentoxyphylline in uterine smooth muscle cells. NO is probably not significantly involved in the relaxing effect of pentoxyphylline, because pentoxyphylline, in the presence of L-arginine, did not significantly affect the degree of contraction inhibition. Because methylene blue antagonised the relaxing effect of pentoxyphylline, we conclude that a signalling pathway that involves cGMP and is independent of NO is possibly involved in the mechanism of its effects on uterine smooth muscle. Our results also suggest that pentoxyphylline could be a potential tocolytic drug.

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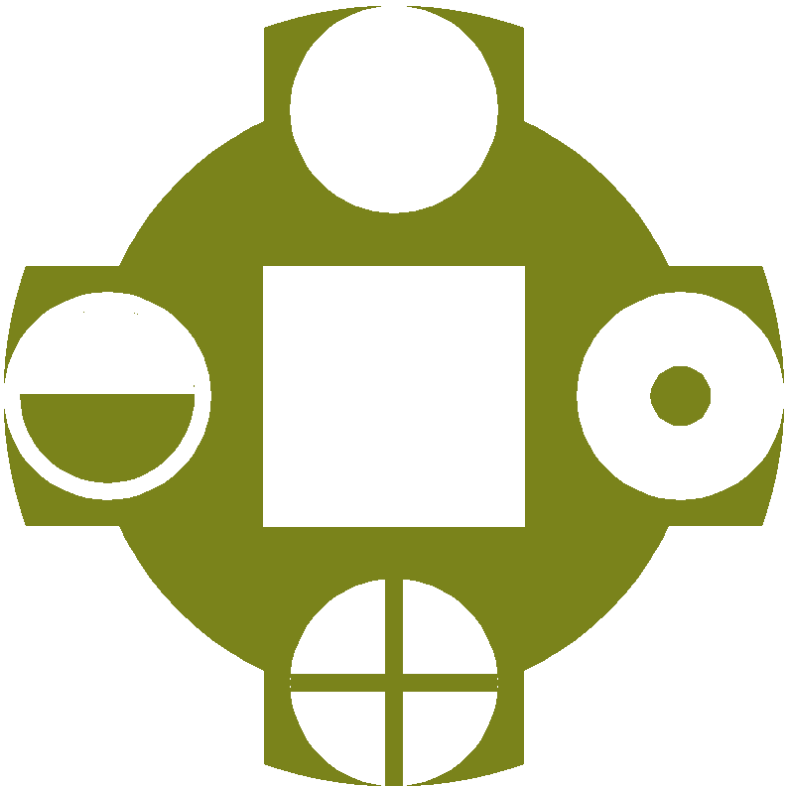

UNILATERAL HYDROSALPINX AND IN VITRO FERTILISATION

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JEDNOSTRANI HIDROSALPINKS I IN VITRO FERTILIZACIJA

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ABSTRACT

We completed a retrospective analysis of laparoscopic procedures (salpingectomy, proximal electrocoagulation of tubes and salpingoneostoma formation) in 30 patients who were treated for fallopian tube infertility. The main cause of infertility was the alteration of the fallopian tubes by a hydrosalpinx. Observed parameters included the following: age, time period and duration of infertility before surgery, number of applied assisted procedures before surgery, number and type of previous operations, number and outcome of previous pregnancies, new outcome and number of realised pregnancies. In the group of examined cases, no male infertility was involved. Applied diagnostic methods included transvaginal ultrasound, laparoscopy and hysterosalpingography.

We categorised the level of the pathological changes of the tubes through clinical examination and complementary technological procedures: (1) a hydrosalpinx is visible with ultrasound before IVF treatment (2) and a hydrosalpinx is visible with ultrasound during IVF treatment (during stimulation), hysterosalpingography and laparoscopy.

The aim of this study was to determine the efficacy of applied laparoscopic procedures and the impact of these procedures on improving results of the treatment of infertility in cases of IVF.

The results and the conclusion are that laparoscopic salpingectomy, because of hydrosalpinx, improves IVF success in patients who undergo this procedure compared with patients who do not have a salpingectomy as a pretreatment for IVF.

Key words: *infertility, hydrosalpinx, laparoscopy, IVF*

SAŽETAK

Urađena je retrospektivna analiza laparoskopskih postupaka (salpingektomija, proksimalna elektrokoagulacija jajovoda i salpingoneostoma) kod 30 pacijentkinja koje su lečene zbog tubarnog uzroka bračne neplodnosti. Osnovni uzrok neplodnosti bili su izmenjeni jajovodi u vidu hidrosalpinksa. Posmatrani parametri su godine starosti, vremenski period i trajanje neplodnosti pre operacije, broj primenjenih asistiranih postupaka pre operacije, broj i vrsta prethodnih operacija, broj i ishod prethodnih trudnoća, novi ishod i broj realizovanih i iznešenih trudnoća. U grupi isptivanih žena nije postojao muški uzrok neplodnosti. Primenjene dijagnostičke metode su transvaginalni ultrazvuk, histerosalpingografija i laparoskopija.

Nivo patološke izmenjenosti jajovoda smo kategorisali kliničkim pregledom i komplementarnim tehnološkim postupcima: hidrosalpinks vidljiv ultzrazvukom pre tretmana IVF, hidrosalpinks vidljiv ultrazvukom tokom tretmana IVF (prilikom stimulacije), histerosalpingografski i laparoskopijom.

Cilj rada je da utvrdimo uspešnost primenjenih laparoskopskih postupaka i njihov uticaj na poboljšanje rezultata u lečenju neplodnosti kod primene IVF-a.

Očekivani reztultati i zaključak su da laparoskopska salpingektomija zbog hidrosalpinksa značajno poboljšava uspešnost IVF u odnosu na pacijentkinje koje nisu imale salpingektomiju kao pretretman IVF.

Ključne reči: *sterilitet, hidrosalpinks, laparoskopija, IVF*

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INTRODUCTION:

A hydrosalpinx is a distally blocked fallopian tube that is filled with serous, mostly clear liquid. Expanded tubes look like sausages, and they can reach a diameter of up to several centimetres.

In the normal function of the fallopian tubes, endosalpinges or cilia are involved in the mobilisation of items towards the uterus, and tube liquid has its own dynamics in the peritoneal cavity where it is further absorbed. If tube fimbria get stuck, it results in obstruction and the accumulation of tube fluid, thus damaging the endometrium by various mechanisms. Then, the tube cannot participate in the reproductive process.

The main cause of distal tube occlusion is pelvic inflammatory disease (PID) as a result of ascending infection, usually by chlamydia and gonorrhoea. All pelvic infections do not cause tube occlusion. Tube tuberculosis is a very rare cause of tube occlusion and hydrosalpinx formation.

Other causes of distal occlusion are adhesive formations from previous surgeries, endometriosis and cancer, which can originate in the tube, ovary or other surrounding organs (1).

Since tube function is blocked, infertility is a common symptom. These infections usually affect both tubes, although changes can often be unilateral. Before infection, tube fluid is sterile and does not contain any infectious agents.

A hydrosalpinx can be diagnosed with a transvaginal ultrasound when the liquid fills, extends and descends in the tubes and shows the typical 'echo lucent' look. Diagnostic methods include the following: transvaginal ultrasound; hysterosalpingography; and laparoscopy, which, in addition to being diagnostic, also prepares the platform for intervention.

In the twentieth century, patients with tube causes of infertility were subjected to corrective surgery (anastomosis of tubes), opening the distal end of the tube (salpingostoma) and the removal of adhesions (adhesiolysis). Unfortunately, the level of pregnancy after these interventions was very low, and the infectious process was constantly returning and damaging the tube, again causing a hydrosalpinx and the formation of adhesions. Ectopic pregnancy is a typical complication of such tube alterations (2).

The reason IVF was originally performed was for tube causes of infertility. The first report from 1994 showed a reduced number of pregnancies and an increased number of abortions for patients with a hydrosalpinx compared with patients with other tube disorders (3).

Today, IVF has become the main treatment for women with a hydrosalpinx who want to become pregnant when male factor infertility is excluded. Several studies show that patients with an untreated hydrosalpinx have a lower level of conception than the control group, and it is thought that tube fluid, which enters into the tube cavity, changes the local environment or affects the embryo (3,4,5).

In recent years, several authors have reported the probability of more successful IVF treatment in cases where unilateral or bilateral salpingectomy has been previously performed. The conclusion was based on nonrandom data. Many data indicate that women with diseases of the fallopian tubes have poor IVF success and a statistically significant benefit of surgical treatment for a hydrosalpinx (6–10).

In cases of extensive inflammatory tube pathology, unilateral or bilateral salpingectomy not only improves IVF results but, in some cases, also reduces the risk of chronic pelvic pain and acute inflammatory processes in response to follicular aspiration or embryo transfer. Among the potential advantages of this procedure, the biggest advantage is the prevention of ectopic ampullary pregnancy. In any case, the risk of intramural ectopic pregnancy still remains after salpingectomy (11).

AIM OF THE WORK AND HYPOTHESES

Our working hypothesis is that unilateral laparoscopic salpingectomy or proximal tube electrocoagulation applied in infertility cases involving a unilateral hydrosalpinx leads to an increase in the number of realised pregnancies.

The aim of our study was to determine whether laparoscopic unilateral salpingectomy or proximal tube electrocoagulation with a unilateral hydrosalpinx increased the success of realised and accomplished pregnancies.

MATERIALS AND METHODS

The research was conducted by examining the documentation of the gynaecology department in the gynaecology and obstetrics services in Health Center Valjevo, as well as by insight into the therapeutic procedures of assisted reproduction in the Special Gynaecology Hospital "Ivanovic" in Belgrade.

The study was approved by the Ethics Committee of the Health Center Valjevo, and all patients signed a consent form, agreeing they were familiar with all aspects of treatment and accepting the appropriate, indicated operational procedure.

The study was retrospective in the form of nonrandom controlled clinical experiments.

The research involved 30 patients.

The criteria for inclusion in the study were the following:

- the presence of unilateral sactosalpinx, verified by transvaginal ultrasound, hysterosalpingography and laparoscopy
- infertility for longer than two years
- previously failed assisted procedure/s with the presence of sactosalpinx
- partner normospermia

Excluded from the study were all patients diagnosed with the following:

- the presence of active infection of the cervical canal and vagina
- the presence of an active urinary tract infection
- the presence of ovarian cysts
- ovarian endometriosis
- uterine myomas located so that they could be a cause of obstruction of tube mobility

All examined women were divided into three groups depending on which type of surgery was done:

- unilateral removal of tubes (salpingectomy) at 5–7 mm from the uterine horns
- electrocoagulation of one fallopian tube at 5–7 mm from the uterine horns with the evacuation of the sactosalpinx contents
- reconstruction of an abdominal mouth of one of the tubes with adhesiolysis

After the patients were informed in detail about the type, benefits and risks of operative treatment, their written consent was obtained for the appropriate operational procedure. According to the protocol of preoperational preparation, all patients were ambulatorily prepared.

The success or failure of surgical procedures for all patients was determined through an analysis of completed pregnancies. The results of biochemical, clinical and ultrasound data of verified pregnancies was compared. Most important, the number of realised pregnancies were analysed. The following were parameters: age, duration of infertility, number and type of previous operations and number and type of previous assisted procedures. The condition of patients' infants were analysed according to Apgar score.

Assisted procedures were attempted 60 days after operative treatment in the period of endometrial renewal.

The health condition of all patients, the way in which pregnancy was achieved, and the type and number of assisted procedures were followed for at least one year after the laparoscopic operations.

Patients who achieved and realised pregnancy were checked until their children's births and again seven days after the births.

Statistics

In the statistical analysis, the following methods were used: descriptive statistics, the relative numbers and the absolute numbers.

RESULTS

Data from 30 patients who had a unilateral hydrosalpinx were analysed.

The average age of these women was 34.8 ± 4.4 years, while the average duration of infertility was 8.0 ± 3.7 years.

The distribution of pregnancies and their outcomes in the period prior to our operation is shown in Table 1.

Table 1

	N	%	% In the group of women who were pregnant
Ectopic pregnancy	11	36.7	55.0
spontaneous abortion	8	26.7	40.0
Spontaneous abortion and ectopic pregnancy	1	3.3	5.0
Total number of women who were pregnant	20	66.7	100.0
Women who were not pregnant	10	33.3	
Total	30	100.0	

Out of 30 patients, 20 of them became pregnant. Eleven patients had ectopic pregnancies, eight had spontaneous abortions, one patient had an ectopic pregnancy and a spontaneous abortion, and 10 patients did not become pregnant.

The distribution of patients with a unilateral hydrosalpinx according to the type of previous operations is shown in Table 2.

Table 2

	H	%	% Of women who have had previous surgery
salpingectomy	8	26.7	57.1
Neostoma formation	5	16.7	35.7
salpingectomy and neostoma formation	1	3.3	7.1
Total number of women who had operations	14	46.7	100.0
Women who did not have operations	16	53.3	
Total	30	100.0	

Of the 14 patients who had operations, 8 had salpingectomies, 5 had salpingoneostoma formations and one patient had both operations. In total, nine salpingectomies were done because of ectopic pregnancies, and three ectopic pregnancies were treated with ampullary methotrexate. Sixteen patients did not have operations.

In addition to the type of previous surgery, the distribution of women with unilateral sactosalpinx by the type of previous assisted procedure has been analysed, and the results are shown in Table 3.

Table 3.

	H	%	% Of women who have had previous assist. procedure
IVF	19	63.3	86.4
IUI	2	6.7	9.1
IVF i IUI	1	3.3	4.5
Total of women who have had previous assist. procedure	22	73.3	100.0
Women who have not had previous assist. procedure	8	26.7	
Total	30	100.0	

Out of the 30 patients, one or both assisted procedures had been completed in 22. IVF was applied in 19 women,

IUI in 2 women, and both IVF and IUI in 1 woman. In eight women, we applied no assisted procedures.

All women were analysed in relation to the type of our operations we applied based on indications and the patient's own wishes, as well as according to treatment outcome.

The distribution of these patients in relation to the type of operation and the outcome of treatment is shown in Table 4.

Table 4.

		The final outcome				Total
		failed fertilisation	spontaneous abortion	Ectopic pregnancy	Realised pregnancy	
Type of the new operations	salpingectomy	2	0	0	15	17
	Electrocoagulation	1	0	0	2	3
	Neostoma formation	2	3	4	1	10
Total		5	3	4	18	30

From a total of 30 patients, 5 had failed fertilisation, 3 had spontaneous abortions and 4 had ectopic pregnancies, while 18 patients had realised pregnancies. In relation to the type of operation, it is evident that the greatest success was with women who had salpingectomies (15 of 17 women) or proximal tube electrocoagulation (2 of 3 women).

DISCUSSION

Tube pathology affects the expression of the endometrial integrity during implantation windows, and in some cases, the expression may be reestablished after removing the pathological tubes (3,12,13). Tube pathology increases the number of macrophages in the endometrium compared with the number of macrophages in the endometria of fertile women. Despite the large number of studies concerning tube pathology with a hydrosalpinx and embryo implantation, the pathophysiology remains unclear (3).

A hydrosalpinx is formed after the destruction of fimbria and, consequently, by the accumulation of different tube secretions. These secretions can reach the uterus. The resulting dysfunction of endometrial receptivity can interrupt the process of embryo implantation. If these hypotheses are correct, bilateral salpingectomy directly affects the cause of endometrial alteration and reestablishes its receptivity (3,12,13). The consequence is the possibility of embryo implantation, which becomes possible after this procedure. The result is spontaneous pregnancy soon after unilateral salpingectomy in hydrosalpinx (3,12,14,15).

In our research, we chose 30 patients who had sactosalpinges with presently severe pathology of their tubes. The patient selections were made after reviewing the internal genital organs with adequate complementary diagnostic procedures: transvaginal sonography; diagnostic laparoscopy; and hysterosalpingography, which is also a platform for the implementation of operational procedures (salpingectomy, proximal tube electrocoagulation with evacuation of the liquid of a hydrosalpinx and salpingoneostoma formation).

Access to surgical treatment was indicated and conditioned, in our opinion, by irreversible pathological changes in the fallopian tubes (sactosalpinx is visible with ultrasound with a diameter of two or more centimetres; it appears as a fallopian tube with severe inflammatory pathology and very thick wall with completely reduced fimbrial device and obliterant mouth with or without the presence of adhesions) and the patient's own consent to the recommended operating procedure.

Other observed parameters were patient age, duration of infertility, number and type of previous operations and assisted procedures, new types of operations, number and type of new assisted process and final outcome.

In many other studies, which had a self-control group, before surgical treatment, the selection for salpingectomy was only based on the number of failures in the application of IVF. These data are taken into account although there was no control group.

Our research was a clinically controlled experiment involving patients whose homogeneity was conditioned by pathological tube substratum, and who are themselves a self-control group.

In this way, optimal conditions were created for observing the desired variables by the type of applied surgical methods.

Due to the high success of the IVF method, which, in all reproductive periods of life, surpasses the performance of spontaneous conception, the indications for ART methods are broad. Among the most important, of course, is emergency IVF, IVF in late reproductive age, and IVF as a substitute for reconstructive surgical techniques of tube infertility treatment in the second half of the reproductive period of life (16).

From a total of 30 patients with unilateral hydrosalpinx, 5 had failed fertilisations, 3 had spontaneous abortions, 4 had ectopic pregnancies and 18 patients had realised pregnancies. In relation to the type of operation, the most success was seen in women who had salpingectomies (15 of 17 women) (Table 4). This result favours the hypothesis of dysfunction of endometrial receptivity and its alteration after the removal of the hydrosalpinx (3,12,14,15).

The largest number of patients had a salpingectomy %), while the tubes were electrocoagulated in the rarest types of operations %). Salpingectomy and proximal electrocoagulation of tubes are procedures that have the same goal, but we performed one or the other according to indications. This type of operation was two times more frequent than formation and is applied only when the patient desires the operational technique. (Table 4).

In all 30 patients, IVF was applied. To patients who required conservation tubes (salpingoneostomas), IVF was also applied by their own choice. The other 25 patients (83.3%) remained pregnant, while 5 patients (16.7%) did not become pregnant. Out of 25 women who became pregnant, 18 women had realised pregnancies (72%), 3 women had complications in the form of spontaneous abortion (12%) and 4 women had ectopic pregnancies (16%).

CONCLUSION

Patients who underwent unilateral laparoscopic salpingectomies or proximal tube electrocoagulations because of unilateral hydrosalpinges as pretreatment for IVF had a greater ability to generate and realise pregnancies compared with patients who used IVF with the hydrosalpinges still present.

After applied laparoscopic procedures before IVF, the number of complications, spontaneous abortions and ectopic pregnancies was significantly reduced.

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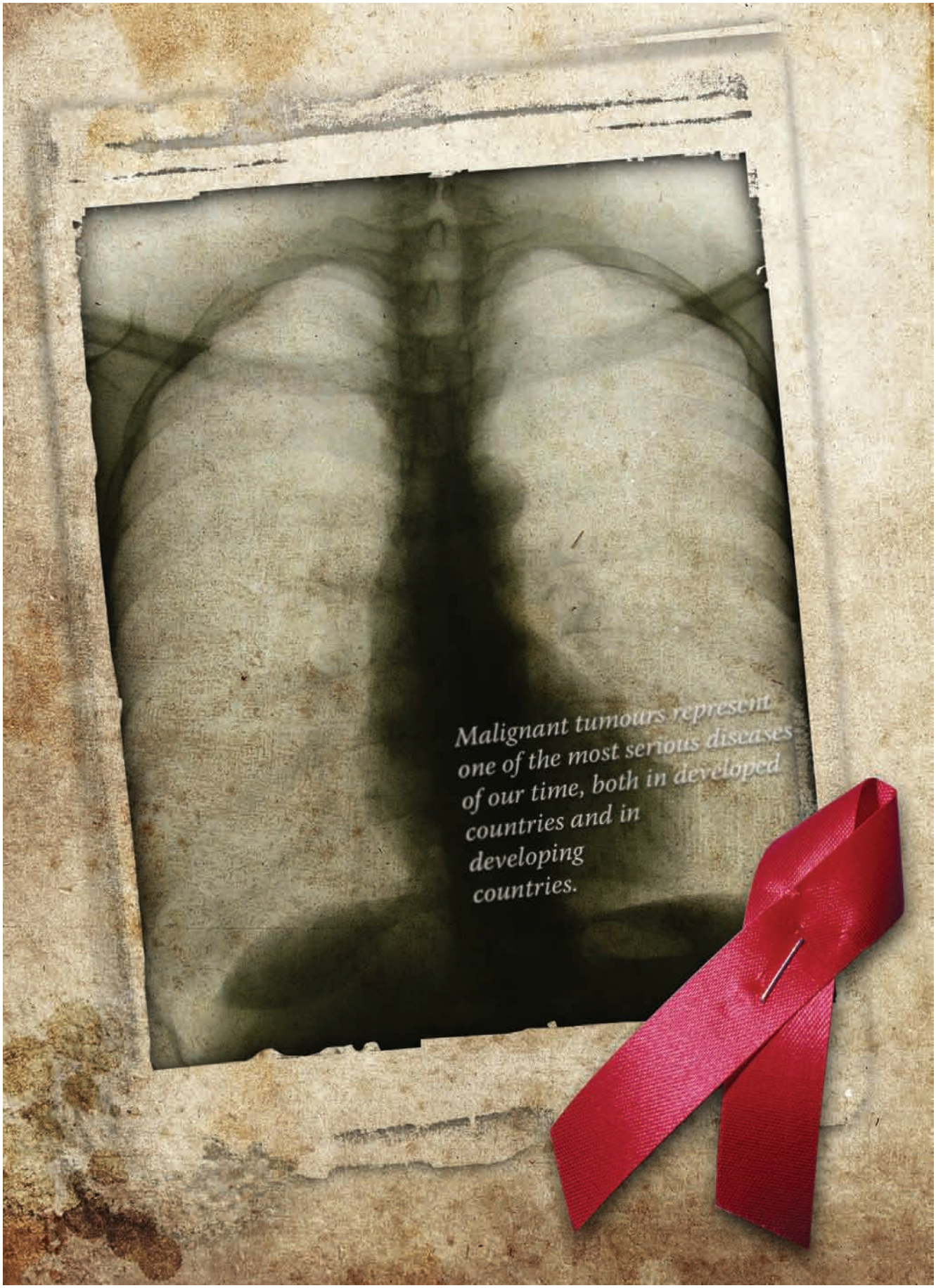
Malignant tumours represent
one of the most serious diseases
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developing
countries.

THE REVISED TNM STAGING SYSTEM FOR LUNG CANCER NON - SMALL CELL LUNG CANCER

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NOVI SISTEM STAŽIRANJA KARCINOMA PLUĆA MEĐUNARODNE ASOCIJACIJE ZA ISTRAŽIVANJE KARCINOMA PLUĆA NEMIKROCELULARNI KARCINOMI PLUĆA (NSCLC)

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ABSTRACT

Malignant tumours represent one of the most serious diseases of our time, both in developed countries and in developing countries. Despite significant advances in prevention, early diagnosis and therapy, mortality from malignant tumours is still very high. The mortality rate associated with lung cancer is the highest among all cancers in men. New diagnostic methods (e.g., positron emission tomography) and innovations in surgical approaches (video-assisted thoracic surgery), radiotherapy (three-dimensional radiotherapy) and chemotherapy (third-generation drugs and targeted therapy) have contributed to improved results in the treatment of lung cancer. A new revision of the international system of staging for lung cancer, which aims to further optimise our approach to this disease, was recently released by the International Association for the Study of Lung Cancer (IASLC).

Key words: *NSCLC, TNM classification, revision*

SAŽETAK

Maligni tumori predstavljaju jednu od najtežih bolesti današnjice, kako u razvijenim zemljama, tako i u zemljama u razvoju. I pored značajnih napredaka u oblasti prevencije, rane dijagnostike i terapije, smrtnost od malignih tumora je i dalje visoka. Karcinom pluća je vodeći među karcinomima po smrtnosti kod muškaraca. Nove dijagnostičke metode (npr. Pozitronska Emisiona Tomografija), noviteti u hirurškom pristupu (Video Assisted Thoracoscopic Surgery -VATS), radioterapiji (trodimenzionalna radioterapija) i hemioterapiji (treća generacija lekova i target - terapija) su doprineli poboljšanim rezultatima u lečenju karcinoma pluća. Nova revizija internacionalnog sistema stažiranja karcinoma pluća je jedna od skorašnjih inicijativa Internacionalne Asocijacije za Istraživanje Karcinoma Pluća (International Association for the Study of Lung Cancer) IASLC) sa ciljem dalje optimizacije naših pristupa ovoj bolesti.

Ključne reči: *NSCLC, TNM klasifikacija, revizija*

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The TNM system, based on classification of primary tumours (T), lymph node metastases (N) and distant metastases (M), has been in use by oncologists since 1953, when the Committee on Nomenclature and Statistics of the International Union Against Cancer (UICC) adopted the TNM system as the basis for the anatomical diagnosis of cancer (1). Since 1973, lung cancer staging up to and including the fifth revision of the staging system made in 1997 has been based on databases taken from surgical series (2). At that time, an international database was created. The IASLC led a coordinated effort lasting more than a decade to find financial support and identify institutions and individuals who would contribute to the realisation of a common international database of patients. Upon completion, this database included 100,869 cases, of which 81,495 cases included adequate time tracking. A total of 68,463 cases were in the non-small cell lung cancer (NSCLC) group, and 13,032 cases were in the small cell lung cancer (SCLC) group (3-8). Of these, surgical treatment was used in only 41% of cases (with radiotherapy for only 11% and chemotherapy for only 23%), whereas the remaining cases were treated by a combined therapeutic approach.

The extremely large number of cases has enabled more analysis, and thus, several sub-committees were formed (focusing on T, N and M indicators, prognostic factors, SCLC, surgical site, validation and methodology). The work of these sub-committees included the analysis of various end results, which were published as a series of articles in the Journal of Thoracic Oncology (3-8), the official journal of the IASLC. The ultimate goal was to revise the TNM classification of lung cancer in January 2009 under the network of the seventh review.

Based on data from 18,198 cases with sufficient data to determine the impact of T stage on NSCLC (3-6), the following changes were made to the staging classification in 1997 (3): T1 tumours are subclassified as T1a or T1b, with a T1a tumour being up to 2 cm in maximal diameter and a T1b tumour being of maximal diameter between 2 and 3 cm. T2 tumours are subclassified into T2a and T2b, with T2 tumours being > 3 cm but ≤ 5 cm in maximal diameter and T2b tumours being > 5 cm but ≤ 7 cm in maximal diameter. Also, tumours bigger than 7 cm in maximal diameter are reclassified as T3 instead of T2, and the T3 category also includes tumours with separate tumour nodule(s) in the same lobe. T4 tumours now include those with separate tumour nodule(s) in a different ipsilateral lobe.

Data from 38,265 cases without clinical evidence of distant metastases (cM0), for which information about the clinical status of lymph nodes (cN) was available, and data from 28,371 surgically treated cases with information on the pathological N stage (pN) were analysed. It was concluded that the existing classification of N stage (N0 - N3) should not be changed.

Regarding metastatic lesions (M+), they are now divided into M1a and M1b, of which the first is defined as a separate tumour nodule (i) in the contralateral lobe, a tumour with pleural nodes, or malignant pleural or pericardial effusion.

Several changes were made to the process of staging based on certain combinations of the descriptors T, N and M. Namely, T2bN0M0 cases were moved from stage IB to stage IIA, and T2aN1M0 cases were moved from stage IIB to stage IIA. Cases categorised as T4N0 - 1M0 were moved from stage IIIB to stage IIIA (Table 1).

Table 1: New (7th) revision of the IASLC staging system.
Grouping of stages:

Occult carcinoma	Tx	N0	M0
Stage 0	Tis	N0	M0
Stage IA	T1a,b	N0	M0
Stage IB	T2a	N0	M0
Stage IIA	T1a,b	N1	M0
	T2a	N1	M0
	T2b	N0	M0
Stage IIB	T2b	N1	M0
	T3	N0	M0
Stage IIIA	T1, T2	N2	M0
	T3	N1, N2	M0
	T4	N0, N1	M0
Stage IIIB	T4	N2	M0
	Any T	N3	M0
Stage IV	Any T	Any N	M1a,b

These changes in staging for NSCLC made by the IASLC and the UICC are the latest contributions to a better understanding of this disease. The staging system, as with other systems for other malignancies, will continue to contribute to an adequate comparison of results between individual institutions and to adequate interpretation of the results of clinical studies in this area by using a "common" language during diagnosis and therapy. This system will also be helpful in making decisions about the optimal treatment of patients and in predicting prognosis after treatment with individual or combined therapeutic modalities. Its immediate application is imperative for thoracic oncologists across the world who wish to further optimise and coordinate efforts in treating patients with NSCLC.

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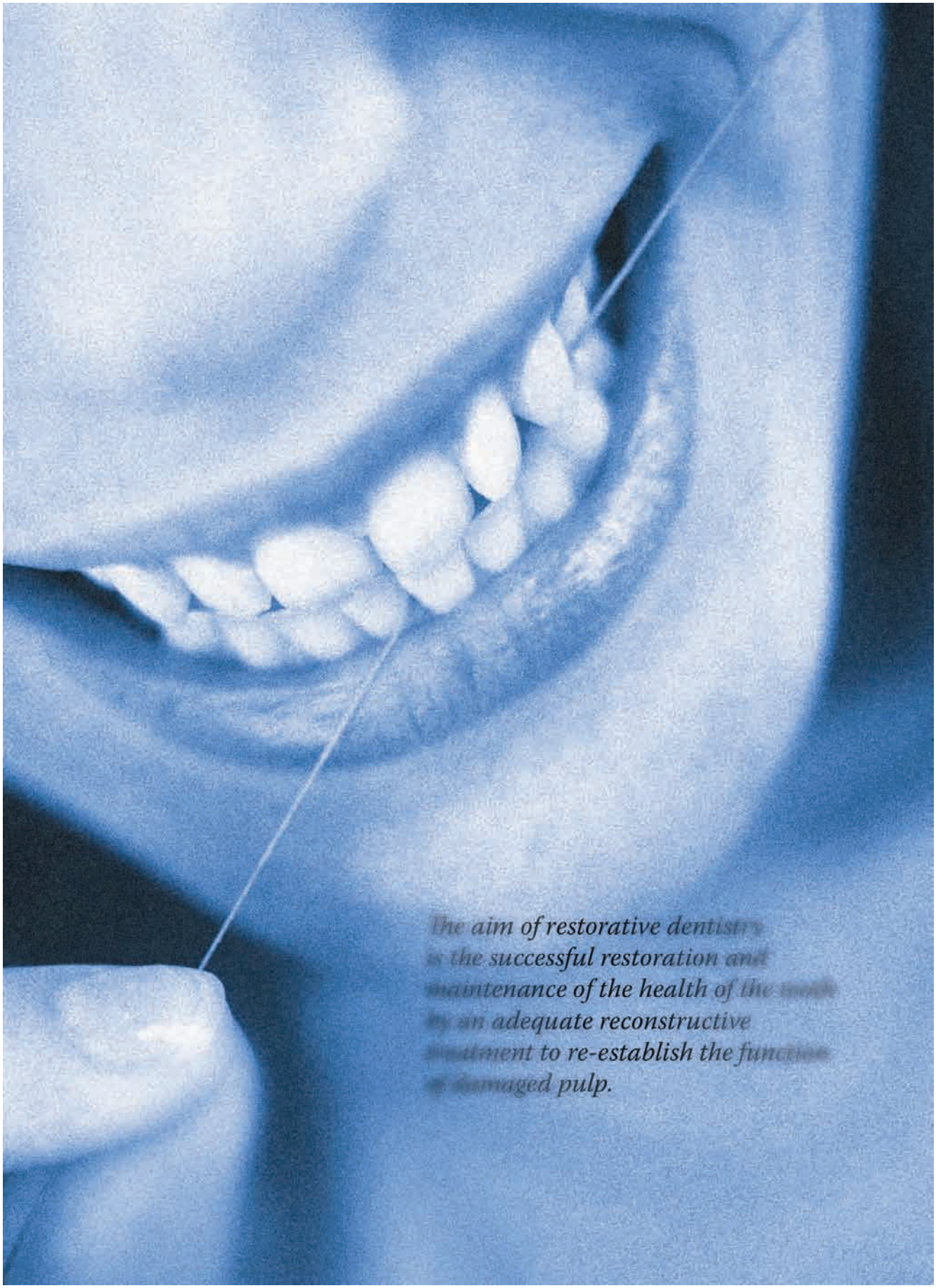
The aim of restorative dentistry
is the successful restoration and
maintenance of the health of the tooth
by an adequate reconstructive
treatment to re-establish the function
of damaged pulp.

CYTOTOXICITY OF GLASS IONOMER CEMENTS ON HUMAN PULP CELLS

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CITOTOKSIČNOST GLAS JONOMER CEMENTA ISPITIVANA NA ĆELIJAMA ZUBNE PULPE LJUDI

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ABSTRACT

The aim of restorative dentistry is the successful restoration and maintenance of the health of the tooth by an adequate reconstructive treatment to re-establish the function of damaged pulp. The potential cytotoxicity of specific materials used in restorative dentistry has been widely studied, and the aim of this review article is to summarise and discuss the cytotoxicity of glass ionomer cements when they are in direct or indirect contact with the pulp tissue. Resin modified and metal reinforced glass ionomer cements, in comparison to conventional glass ionomer cements, showed higher cytotoxic effects on pulp cells in vitro. In vivo, the dentin barrier between toxic glass ionomer cements and the pulp cells may prevent pulp cell damage. Potentially toxic resin modified and metal reinforced glass ionomer cements should not be applied directly to the pulp tissue.

Keywords: *cytotoxicity, pulp cells, glass ionomer cements*

SAŽETAK

Čuvanje funkcije zubne pulpe je važno za zdravlje zuba. Cilj ovog preglednog rada je predstavljanje do danas poznatih rezultata in vitro i in vivo studija koje su ispitivale citotoksičnost glas jonomer cementa, materijala koji se koriste u stomatologiji i koji su u direktnom ili indirektnom kontaktu sa zubnom pulpom., „Smolom modifikovani" i, „metalom ojačani" glas jonomer cementi, su in vitro pokazali znatno veću citotoksičnost, u poređenju sa konvencionalnim glas jonomer cementima. In vivo, dentin predstavlja barijeru koja štiti ćelije zubne pulpe od toksičnog dejstva glas jonomer cementa i zato potencijalno toksične „smolom modifikovane" i „metalom ojačane" glas jonomer cemente, nikada ne treba direktno aplikovati na zubnu pulpu.

Ključne reči: *citotoksičnost, ćelije zubne pulpe, glas jonomer cementi*

INTRODUCTION

The aim of restorative dentistry is the successful restoration and maintenance of the health of the tooth by an adequate reconstructive treatment to re-establish the function of damaged pulp (1).

The main role of pulp is dentin formation and nutrition as well as the innervation of teeth. The primary function of pulp is dentin formation, which begins when the mesenchymal cells differentiate into odontoblasts and ends when the tooth is completely formed. The channels created by the odontoblasts function in dental nutrition; the continuous transport of nutrients and fluids maintains the vitality of the pulp. Throughout an individual's lifetime, pulp continuously produces dentin in physiological condition as well as in response to physical and chemical injuries. Pulp also serves as a defence barrier. Increased dilatation and permeability of blood vessels and intensive migration of inflammatory cells are usually results of pulp response to different noxious stimuli (2).

In restorative dentistry, the protection of the dentin-pulp complex consists of the application of one or more layers of specific materials (e.g., varnishes, calcium hydroxide-based products, glass ionomer cements (GICs) and adhesive systems) between the restorative material and dental tissue to avoid additional damage of pulp tissue caused by operative procedures, toxicity of restorative materials and bacterial penetration due to microleakage (1, 3).

The potential cytotoxicity of the specific materials used in restorative dentistry has been widely studied, and the aim of this review article is to summarise and discuss the cytotoxicity of GICs when they are in direct or indirect contact with the pulp tissue.

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THE CHARACTERISTIC AND CLASSIFICATION OF GLASS IONOMER CEMENTS

Glass ionomer cements (GICs), invented and originally described by Wilson and Kent (4), consist of a basic glass powder (calcium or strontium aluminofluorosilicate) and a water-soluble acidic polymer, such as polyacrylic acid (5). GICs are classified into three categories: conventional, metal-reinforced and resin modified [6-7]. Metal-reinforced GICs are strengthened by the inclusion of finely divided metal powders, typically the silver-tin alloy of dental amalgams (8).

Although conventional GICs, because of their similarity to dentin in terms of biocompatibility, elasticity and ability to release fluoride, have advantages in comparison with other materials used in restorative dentistry, they have several limitations, such as susceptibility to dehydration and poor physical properties (i.e., high solubility and slow setting rate) (1, 9-10).

The incorporation of polymerisable water-compatible monomers such as 2-hydoxyethyl methacrylate (HEMA) led to the introduction of hybrid versions of conventional GICs named resin-modified GICs (RMGICs) (1, 8). In comparison with conventional GICs, RMGICs show enhanced flexural strength, diametral tensile strength, elastic modulus and wear resistance, but they are not as biocompatible as conventional GICs (11).

THE CYTOTOXIC EFFECTS OF GLASS IONOMER CEMENTS

The main disadvantage of metal-reinforced GICs and RMGICs is their higher cytotoxicity in comparison with conventional GICs (1, 12).

The cytotoxicity of RMGICs could be due to their constituent HEMA which, because of their hydrophilicity and low molecular weight, can easily diffuse through the dentinal tubules; reach dental pulp cells; damage pulp cells by suppressing cell growth and proliferation; and induce apoptosis of dental pulp cells (12).

It has been shown that methacrylate monomers present in resin based materials such as Vitrebond (3M/ESPE Dental Products, St. Paul, MN, USA) and Vitremer (3M/ESPE Dental Products, St. Paul, MN, USA) are incorporated in the lipid bilayers of cell membranes that can cause dysfunction of cellular membranes and consequently induce cell death (13). This was documented by Costa et al. in a study in which the cytotoxicity of several GICs was tested on an odontoblast cell line (MDPC-23) by a 3-(4, 5-dimethyl-thiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide (MTT) assay (13). It was shown that two GICs, Fuji IX GP (GC, Tokyo, Japan) and Ketac Molar (3M/ESPE Dental Products, St. Paul, MN, USA), were the least cytotoxic among the tested materials while two other RMGICs, Vitrebond and Vitremer, caused intense cytopathic effects on the cultured cells by significantly decreasing cell metabolism and by causing remarkable cell death (13). The highly cytotoxic effects of Vitremer on cultured human osteoblastic cells was confirmed by Oliva et al. (14), who showed *in vitro* that HEMA is mainly responsible for the cytotoxicity of Vitremer.

To compare the cytotoxic effects of different RMGICs and metal reinforced GICs, Stanislawski et al. carried out an *in vitro* pulp cell viability assay in which metal reinforced GICs were shown to be highly toxic materials. Vitremer was the most toxic material among the RMGICs while the least toxic were Compoglass (Ivoclar Vivadent Ltda., São Paulo, SP, Brasil) and Photac Fil (3M/ESPE Dental Products, St. Paul, MN, USA) (11).

It was documented that HEMA, along with other unpolymerised monomers like triethylene glycol dimethacrylate (TEGDMA), is responsible for the cytotoxic effects of RMGICs and metal reinforced GICs.

However, the presence of some ions in significant amounts in metal reinforced GICs and also in RMGICs also could be responsible for their cytotoxicities. The ions Cu2+ and Ag+, which were present in toxic concentrations, could be the main elements responsible for the toxicity of the metal-reinforced GICs along with HEMA and TEGDMA (11). Further, the possible cytotoxic effects of F-, Al3+, Zn2+ and Sr2+ ions, which are also present in significant amounts in GICs, were tested. It was concluded that among the tested ions, only the zinc ion was found to be associated with a high enough concentration to induce cytotoxicity of metal reinforced GICs and RMGICs (11).

Conversely, Soheili Madj et al. demonstrated that the cytotoxic effects of GICs might be caused by the metal components, or the small amounts of aluminium and/or iron ions present in their composition, which may cause cytotoxic effects on cultured cells by oxidative stress (15).

The potential toxic effects of the organic components of GICs also have been tested. Leyhausen et al. suggested that the cytotoxicity of Vitrebond may be caused by chlorine benzene, iodine benzene, and bromide benzene, which are decomposition products of the initiator diphenyliodonium chloride (DPICl) (16).

Complementing the results presented above, the RMGICs and metal reinforced GICs showed higher cytotoxic effects on cultured fibroblasts and osteoblasts *in vitro* in comparison with conventional GICs. It has been shown that RMGICs are able to cause intense cytophatic effects on the cultured cells by significantly decreasing cell metabolism as well as by causing remarkable cell death (1).

However, it is most important to point out that the toxic effects of GICs were tested mainly *in vitro*. Although *in vitro* tests are simple to perform, cost-effective and suitable as an alternative to in vivo experiments, the results of *in vitro* studies cannot be automatically extrapolated to clinical situations (17-19).

It has been shown that the sensitivity of human pulp cells to cytotoxicity depended on the differences in the content, specifically the component monomers or additives of the tested RMGICs. Additionally, the sensitiv-

ity of human pulp cells to cytotoxicity depended on the concentration of the elutes tested. The cytotoxicities of the tested RMGICs decreased as the dilution concentration of the elutes increased because an increase of dilution concentration of the elutes is followed by a decrease in the content of the monomers or the additives in the dilution (17).

In line with this observation are the results of in vivo studies performed in human teeth, which have demonstrated that the RMGIC Vitrebond, the GIC which showed toxic effects *in vitro*, caused no inflammatory pulp response when it was applied in vivo as a liner in very deep class V cavities, (18-19). It seems that pulp cell damage documented in *in vitro* studies is prevented in vivo by the presence of a dentin barrier between the RMGIC Vitrebond and the pulp cells.

CONCLUSION

Based on this literature review, it may be concluded that RMGICs and metal reinforced GICs, in comparison with conventional GICs, showed higher cytotoxic effects on pulp cells *in vitro*. Monomers (HEMA and TEGDMA) present in resin composites of RMGICs, as well as Cu2+, Ag+ and Zn2+ ions, present in significant amounts in metal reinforced GICs and are the components mainly responsible for the toxic effects of these GICs.

In vivo experiments showed that the presence of a dentin barrier between toxic GICs and the pulp cells may prevent pulp cell damage. Potentially toxic RMGICs and metal reinforced GICs, because of their cytotoxicity, should not be applied directly to the pulp tissue.

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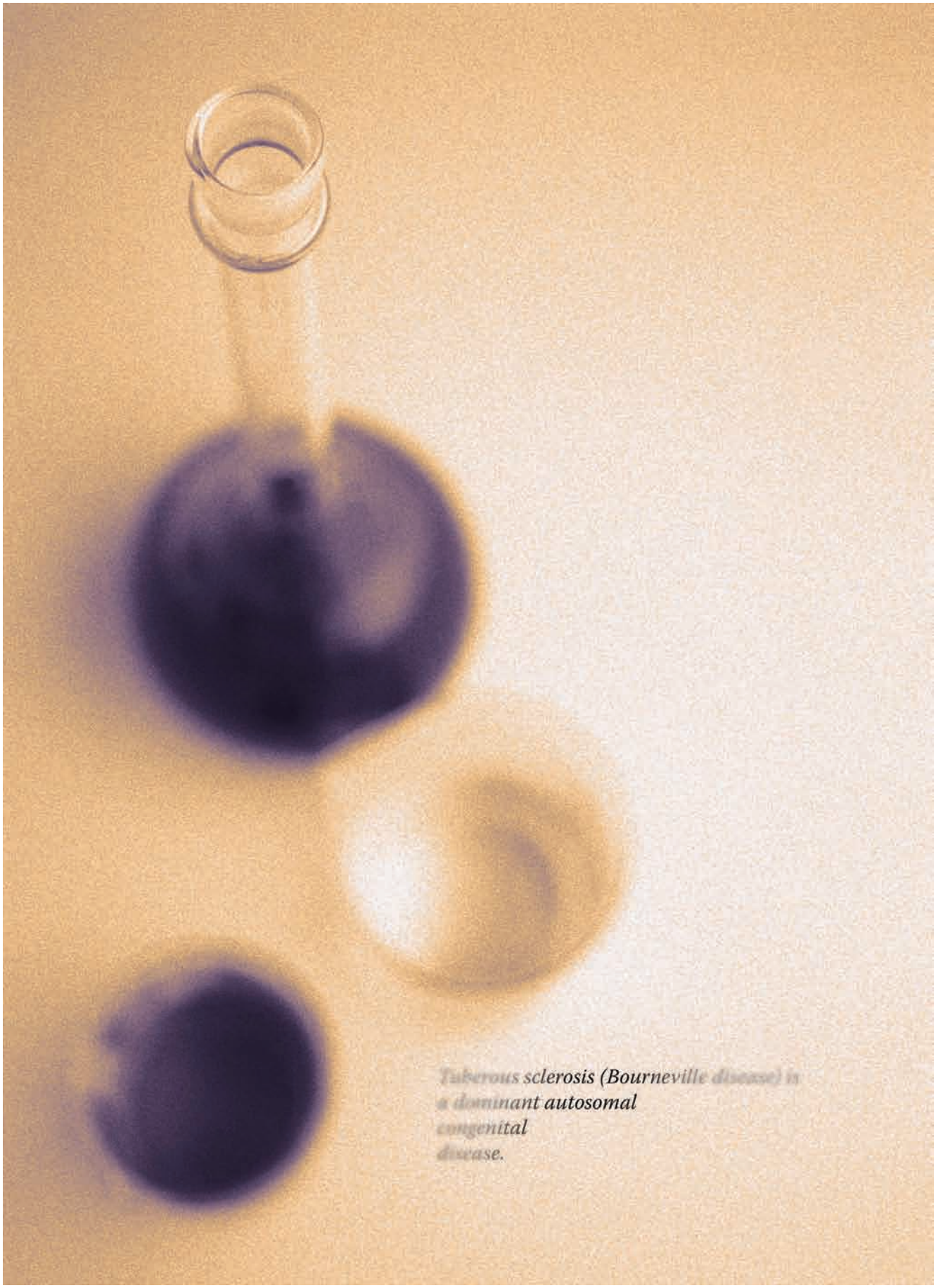

Tuberous sclerosis (Bourneville disease) is a dominant autosomal congenital disease.

BILATERAL RENAL ANGIOMYOLIPOMA IN A PATIENT WITH TUBEROUS SCLEROSIS – CASE REPORT

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BILATERALNI RENALNI ANGIMIOLIPOM KOD PACIJENTA SA TUBEROZNOM SKLEROZOM-PRIKAZ SLUČAJA

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ABSTRACT

Tuberous sclerosis (Bourneville disease) is a dominant autosomal congenital disease. It is caused by alterations in the ninth or sixteenth chromosome and normally is characterised by a classical triad of mental retardation, epilepsy and sebaceous adenoma. The radiological diagnosis is made by observing some combination of findings: subependymal and periventricular calcifications, cortical tubers, cardiac tumours, renal cysts, and angiomyolipomas. Renal angiomyolipoma (AML) is a benign renal neoplasm, which is composed of fat as well as vascular and smooth muscle elements. AMLs occur in association with tuberous sclerosis in 20% of cases, and they are larger and bilateral, and they affect a younger age group. We report on the case of a 52-year-old woman with abdominal pain, vomiting and anaemia. The initial ultrasound of the abdomen showed bilateral, large, heterogenic, echogenic masses that occupied the renal fosses, and the kidneys were not observable. The patient was hospitalised for observation and additional radiological diagnostic. The diagnosis of tuberous sclerosis with bilateral renal AML was made based on the presence of typical skin lesions and findings on abdominal and head CT as well as intravenous urography. Currently, despite large bilateral renal AML, her laboratory results showed mild anaemia without renal failure, so the patient has not required surgery.

Key words:
angiomyolipoma, tuberous sclerosis, kidneys

SAŽETAK

Tuberozna skleroza (Bourneville bolest) je autozomalno dominantno nasledno oboljenje. Nastaje kao rezultat alteracija na devetom i šesnaestom hromozomu i najčešće se karakteriše trijadom koja obuhvata mentalnu retardaciju, epilepsiju i prisustvo sebaceoznih adenoma. Dijagnoza se postavlja kombinacijom radioloških nalaza koji uključuju prisustvo paraventrikularnih i subependimalnih kalcifikacija, kao i kortikalnih tubera u mozgu, kardioloških tumora, renalnih cista i angimiolipoma. Ranalni angimiolipom (AML) je benigni tumor bubrega, koji se sastoji od masti, vaskularnih elemenata i glatkih mišićnih vlakana. Kod bolesnika sa tuberoznom sklerozom renalni AML se javlja u 20% slučajeva i u tom slučaju je obično bilateralni, veći i zahvata mladju populaciju, nego kad se javlja samostalno. Predstavljamo slučaj pacijentkinje stare 52 godine kod koje se javlja abdominalni bol, mučnina i anemija. Inicijalno, ultazvuk je pokazao ogromnu, bilateralnu, hiperehogenu masu koja se nalazila u projekciji bubrega, koji nisu mogli biti diferencirani. Pacijentkinja je hospitalizovana u cilju observacije i dodatne radiološke dijagnostike. U skladu sa nalazima kompjuterizovne tomografije abdomena i glave, intravenske urografije, kao i zbog postojanja tipičnih promena na koži, dijagnoza tuberozne skleroze sa renalnim angimiolipomom je postavljena. Medjutim, uprkos ogromnim bilateralnim angimiolipomima bubrega, laboratorijski nalazi su ukazivali na blagu anemiju, bez poremećaja bubrežne funkcije,te pacijentkinji nije bila neophodna hirurška intervencija.

Ključne reči:
angimiolipom, bubrezi,tuberozna skleroza

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INTRODUCTION

Tuberous sclerosis complex (TSC) is a rare, inherited autosomal dominant multisystem disorder characterised by the potential for the presence of hamartomas (1). Molecular genetic studies have identified two loci for TSC; TSC1 is located on the long arm of chromosome 9 (9q34); TSC2 is located on the short arm of chromosome 16 (16p13.3). Both TSC1 and TSC2 have tumour suppressor activity that, when not activated, leads to uncontrolled cell cycle progression and the proliferation of hamartomas throughout the body (2).

A hamartoma is a benign tumour comprised of an overgrowth of mature cells and tissues that normally occur in the affected tissue, but typically, one element is predominant.

Tuberous sclerosis symptoms include adenoma sebaceum of the skin, angiomyolipomas of the kidney, cortical and subependymal tubers of the brain, rhabdomyomas of the heart and pulmonary lymphangioleiomyomatosis (LAM). Individual hamartomas are rare in the non-TSC population, so the presence of hamartomas in two different organ systems is considered by some clinicians to be sufficient for the diagnosis (3).

Dermatological Manifestations. The most common dermatological manifestations are hypomelanotic macules or "ash leaf spots" (4). Typically, these lesions are difficult to visualise without the aid of an ultraviolet light (Wood's lamp). Hypomelanotic macules usually become more apparent with age. Facial angiofibromas (adenoma sebaceum) are composed of vascular and connective tissue elements and typically appear on the face as small pink to red dome-shaped papules in a "butterfly distribution." The lesions enlarge gradually and increase in number with age. Periungual and ungual fibromas (Koenen tumours) are smooth, firm, nodular or fleshy lesions that are adjacent to or underneath the nails (5). Toenails are more commonly involved than fingernails (4, 6). Café au lait spots are seen in up to 30% of patients with TSC (7).

A thorough dermatological examination is essential because many TSC's major features are cutaneous. If hypopigmented macules are not obvious under ambient light, a Wood's lamp illumination of the skin should be done for every child with infantile spasms, cardiac rhabdomyoma, or renal angiomyolipoma.

Neurological Manifestations. The neurological manifestations are heterogeneous. The spectrum of manifestation ranges from patients with normal intellect and no seizures to those with severe mental retardation and incapacitating seizures. Neurological complications are the most common cause of mortality and morbidity as well as the most likely to affect the quality of life. The most common seizure types are infantile spasms, partial motor seizures, and generalised tonic-clonic seizures (8, 9). Autism, attention deficit, hyperactivity, and sleep problems are the most frequent behavioural disorders (8, 10, 11). The intracranial abnormalities include tubers, subependymal nodules, and subependymal giant cell astrocytomas (12). No correlation has been found between the number of subependymal lesions and the clinical severity of TSC (13).

Renal Manifestations. Renal complications are the second most common cause of mortality (14). The most common renal lesion is an angiomyolipoma, which occurs in approximately in 80% of cases. Angiomyolipomas are benign tumours comprised of blood vessels with thickened walls, immature smooth muscle cells, and adipose tissue (14, 15). Angiomyolipomas in tuberous sclerosis are larger than the isolated variety, often occur as multiples, are often bilateral, and affect an earlier age group (16, 17). Smaller angiomyolipomas usually do not cause symptoms, but lesions larger than 4 cm in diameter are associated with an increased risk of serious haemorrhage (5, 15). The second most common renal manifestation is a renal cyst, and renal cysts combined angiomyolipomas are characteristic of TSC (5). Renal carcinomas are rare and tend to grow more slowly in patients with TSC than in those found in the general population (15).

Cardiac Manifestations. Cardiac rhabdomyomas usually occur in multiples and are asymptomatic (5, 9). However, these lesions can result in an outflow obstruction, valvular dysfunction, arrhythmias (especially Wolff-Parkinson-White syndrome), and cerebral thromboembolism (13, 18).

Ophthalmic Manifestations. Retinal hamartomas are bilateral and asymptomatic, but some patients have visual impairment as a result of a large macular lesion (13). Angiofibromas may develop on the eyelids (19).

Oral Manifestations. Gingival fibroma occurs in 50% of adults with TSC (19).

Vascular Manifestations. Patients with TSC are at increased risk for arterial aneurysms, which affect the aorta and peripheral arteries (e.g., carotid, renal, intracranial) with potentially appreciable morbid or mortal consequences (18, 19). Histologically, the arterial walls demonstrate a loss of elastin fibres similar to that seen in patients with Marfan Syndrome (18).

Pulmonary Manifestations. The classic pulmonary lesion is lymphangioleiomyomatosis (LAN), a progressive lung disease seen mainly in adult females (20).

Osseous Manifestations. Osseous lesions on radiographs include bone cysts found mainly in the phalanges of the hands and feet, sclerotic lesions, and periosteal new bone formation (21).

Gastrointestinal Manifestations. Hamartomatous polyps in the rectum are common and are usually asymptomatic (4).

RADIOLOGY FINDINGS

Abnormal radiological findings are important in diagnosing this disease and include lesions found in the CNS, heart, lungs, kidneys, skeleton, and, occasionally, liver, spleen and pancreas.

CT findings

Overall, CT reveals intracranial abnormalities in 85% of patients with tuberous sclerosis. CT readily depicts calcified cortical tubers and calcified subependymal nodules. The frequency of their calcification increases with patient age. Subependymal nodules are found mostly along the lateral ventricles. These nodules may enhance after the intravenous administration of contrast material, but contrast enhancement is more difficult to recognise on CT scans than on other images, particularly in calcified lesions. In 10%-15% of patients, subependymal nodules may transform into giant cell astrocytomas. These tumours are benign and usually occur at or near the foramen of Monro. These lesions typically appear inhomogeneous and usually have an inhomogeneous enhancement pattern after the intravenous administration of contrast material. Frequently, they are calcified, they usually enlarge over time, and they commonly cause obstructive hydrocephalus.

Renal angiomyolipomas often have low attenuation values if they contain sufficient fat, but they are indistinguishable from other renal tumours if they contain little or no lipids. Varying amounts of non-lipid tissue and haemorrhage can be visualised on CT scans of angiomyolipomas. Generally, calcification is not seen in angiomyolipomas. Cystic lesions commonly occur for this disease, and they are well characterised with CT. Multiple cysts can distort the renal collecting system; with this finding alone, tuberous sclerosis is indistinguishable from polycystic kidney disease.

ULTRASOUND findings

On sonograms, the lesions are highly echogenic because of their high fat content. A finding of multiple angiomyolipomas with a high fat content is highly suggestive of tuberous sclerosis. Angiomyolipomas can bleed and cause renal parenchymal haemorrhage as well as subcapsular or retroperitoneal haemorrhage. Cysts almost always occur in multiples; typically, they are bilateral, and on sonograms, cysts are anechoic.

CASE REPORT

A 52-year-old woman at a medical check-up presented with a dull, temporary pain in both flanks that radiated to the bladder, but she had no renal failure. History was significant for one year earlier.

The initial abdominal ultrasound examination showed a large, hyperechogenic mass that occupied the right abdomen and was compressible, which implied intestine. A similar structure was seen in the left abdomen, posteriorly and paravertebrally. The kidneys were not seen either by frontal or by posterior positioning of the sonde. The patient was prescribed additional radiological and laboratory diagnostic and surgical examinations (figure 1).

Abdominal palpation revealed bilateral tender flank masses extending up to both iliac fosses that were insensible on palpation and lacked peritoneal irritation. They each had a smooth surface of solid consistency, low mobility and clear limits. The patient reported frequent urination without haematuria and temporary pain in both flanks.

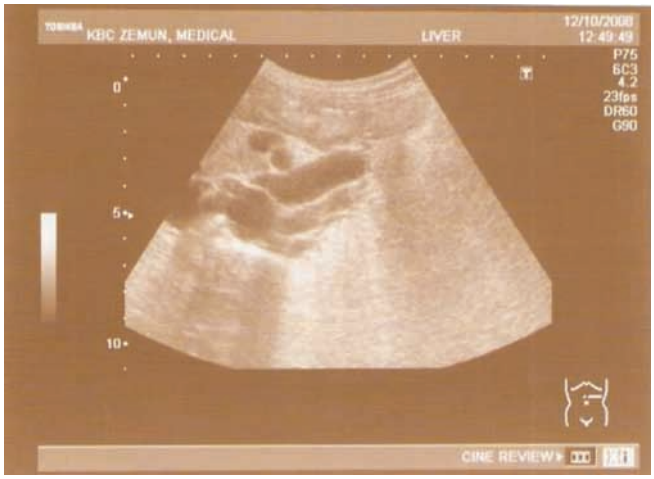

Figure 1. An abdominal ultrasound shows a hyperechogenic mass under the liver in the right abdomen that compresses, which implies intestine.

Dermatological consultation revealed erythema on the forehead, a paranasal rash with multiple sebaceous adenomas around the nose and nasolabial folds (figure 2), some hypomelanotic macules, and fibromatous tumours (Koen) under the toenails with disfiguration of the nails.

Laboratory analysis revealed mild anaemia (RBC 2, 12; Fe 6, 5; Hgb 96) with normal levels of creatinine and urea (Cre 89; Urea 5, 8) that suggested the renal profile was normal.

IMAGING FINDINGS:

Abdominal contrast-enhanced CT revealed a large tumour in the retroperitoneum with primarily fat-equivalent attenuation (image density was -50 HU) that completely replaced both of the kidneys. Intraperitoneal structures

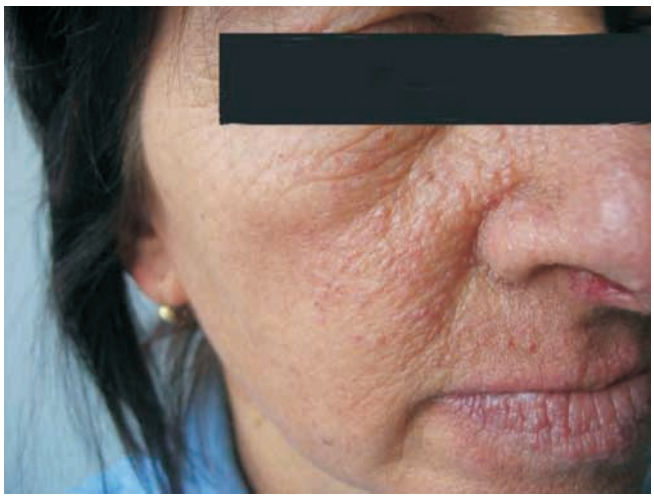

Figure 2. Erythema on the forehead, paranasal rash, and multiple facial angiofibromas around the nose and nasolabial folds are dermatological manifestations.

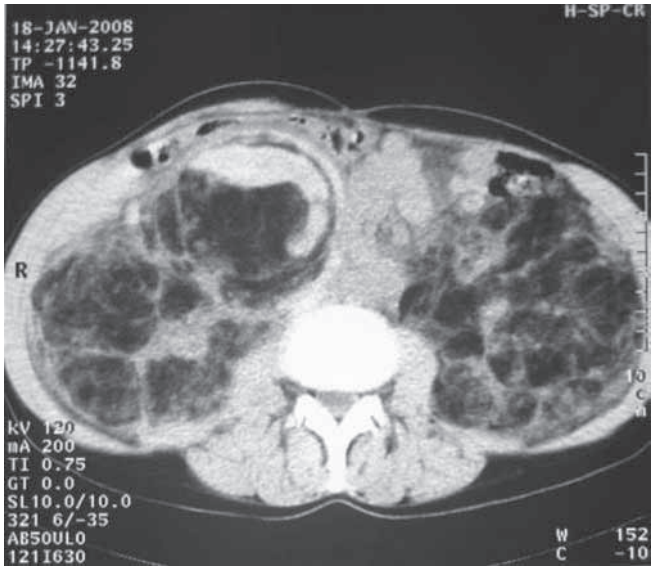

Figure 3. As seen on the abdominal CT, there is a large tumour in the retroperitoneum with predominantly fat-equivalent attenuation that replaced both the kidneys entirely and displaced intraperitoneal structures anteriorly.

were displaced anteriorly, and the mass extended as far as the pelvic region (figure 3).

The head CT revealed cortical and subcortical reductive alteration: multiple calcified subependymal and paraventricular tubers, subcortical calcified nodules in the left hemisphere of the cerebellum and benign white matter lesions (figure 4).

The chest CT revealed bilateral, symmetrical, and diffuse distribution of small air cysts with thin regular walls and normal lung parenchyma between the cysts.

Intravenous urography showed a compressed and extended pyelic system of both kidneys. Kidney structure was not discernable. The contrast was excreted, and the ureters were visualised bilaterally. The bladder was normal (figure 5).

Echocardiography and chest x-ray revealed no abnormalities.

Based on these examinations, the diagnosis was Bourneville-Pringle syndrome, tuberous sclerosis. Despite the bilateral large retroperitoneal angiomyolipoma of the kidneys, the patient's condition was satisfactory, and the renal profile was normal. For that reason, there will further observation without surgery.

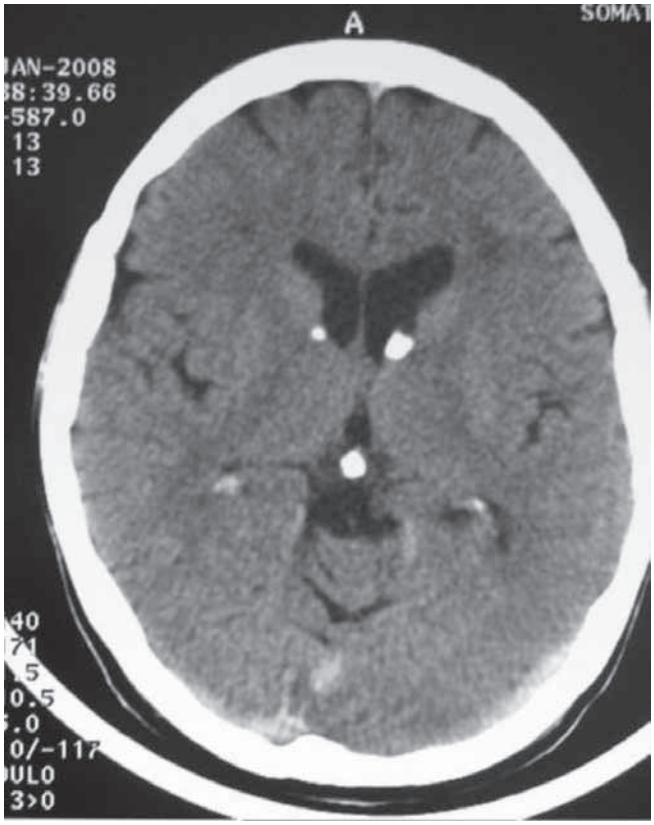

Figure 3. As seen on the abdominal CT, there is a large tumour in the retroperitoneum with predominantly fat-equivalent attenuation that replaced both the kidneys entirely and displaced intraperitoneal structures anteriorly.

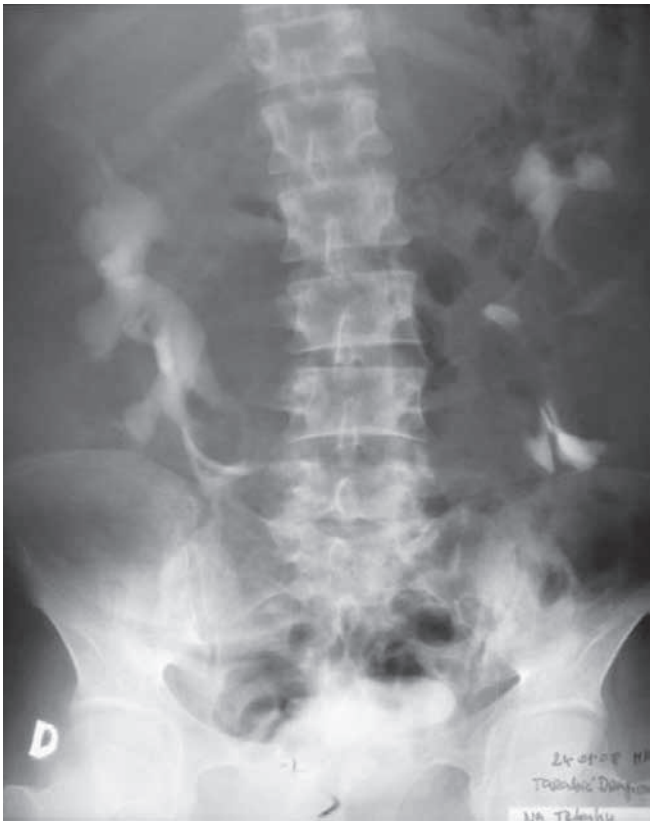

Figure 3. As seen on the abdominal CT, there is a large tumour in the retroperitoneum with predominantly fat-equivalent attenuation that replaced both the kidneys entirely and displaced intraperitoneal structures anteriorly.

DISCUSSION

Angiomyolipomas are uncommon, benign, renal neoplasms comprised of blood vessels with thickened walls, immature smooth muscle cells and mature adipose tissue. They occur as isolated, sporadic entities in 80% of cases. The remaining 20% develop in association with tuberous sclerosis (16, 17). This tumour is one of the major diagnostic criteria of tuberous sclerosis (16). The occurrence of angiomyolipomas in the tuberous sclerosis complex can be up to 80%. Although the histological appearance of angiomyolipomas in these two entities is identical, renal angiomyolipomas with tuberous sclerosis are distinctly different from those without tuberous sclerosis. Angiomyolipomas in association with tuberous sclerosis manifest at a younger age. They are likely to be larger and bilateral and are prone to grow and need surgical treatment (17). In the series by Steiner et al., the average size of AML in patients with TS is 9.6±4.8 cm and 4.1±3.4 cm in those without TS (17) (Table 1).

Angiomyolipoma – benign mesenchymal tumour	
ISOLATED (80%)	ASSOCIATED WITH TS (20%)
- usually solitary - unilateral (80% on the right side) - not associated with TS - mean age of incidence: 40-49 years - much more common in females	- occurs in 80% of patients with TS - commonly large - usually bilateral - usually multiple - may be only evidence of TS - mean age of incidence: 12-19 years - equal incidence in males and females

Table 1. Types of angiomyolipomas. Differences between the isolated form and the form associated with TS.

The hormonal influences of the steroid receptors in the muscle cells may explain the differences in tumour behaviour seen during different periods of life, with greater tumour growth in postpubertal period and during pregnancy (22). L'Hostis et al. observed the presence of both progesterone and oestrogen receptors in angiomyolipomas, and they found that progesterone and oestrogen immunoreactive angiomyolipomas were predominantly found in women and in patients with tuberous sclerosis (23). These findings may further explain the more aggressive nature of the disease process in patients with tuberous sclerosis, the hormonal potentiation of tumour growth and haemorrhage in conditions such as pregnancy, and the overwhelming female predominance in the sporadic form of angiomyolipoma without tuberous sclerosis (23, 24-26).

Angiomyolipomas remain silent and are commonly incidental findings, but they may manifest with symptoms of abdominal and flank pain, gross haematuria, nausea, vomiting, fever, abdominal distension or simply as a mass (16, 17, 27, 28). Common findings include a palpable mass, abdominal tenderness, haematuria, anaemia, shock, hypertension, urinary tract infections, and renal failure. These signs and symptoms are usually a result of the effects of the mass and haemorrhage (16). The propensity to haemorrhage is related to multiple factors: focal deficiencies of elastic tissue in abnormally rigid and thick blood vessels, hypervascularity, and venous invasion (29-31). The defects in the vessel's elastic tissue also predispose these lesions to develop aneurysms. Our patient presented late with abdominal pain and mild anaemia but no renal failure.

With advances in cross-sectional imaging, the diagnosis of renal angiomyolipoma can usually be made confidently without surgery. The demonstration of fat on renal ultrasound and CT can accurately diagnose angiomyolipoma in 95% of cases (16). Angiomyolipomas are well-defined hyperechoic masses based on ultrasonography (US) regardless of the relative fat component (32). Angiomyolipoma fat is easily identified on CT, which helps to make the radiological diagnosis. Demonstration of intratumoural fat attenuation is almost pathognomonic for this lesion (16, 17, 27, 28). Single or multiple well-circumscribed renal cortical tumours containing tissue with fat attenuation of less than -20 HU are characteristic findings of angiomyolipoma on non-enhanced CT (16). In the present case, a diagnosis of AML was made based on typical ultrasound and CT findings.

Angiomyolipomas are at risk for spontaneous haemorrhage. Lesions greater than 4 cm are at greater risk of serious spontaneous haemorrhage and need to be evaluated. Tumour diameter >4 cm has also been used as a criterion for prophylactic treatment because many studies show a higher frequency of haemorrhagic complications with larger tumours (17, 33). However, according to Antonopoulos et al. (34), these tumours do not have to be large (>4-cm diameter) before serious life-threatening haemorrhage can occur, as previous studies have suggested, and at least one study has shown that smaller tumours <4 cm have a more rapid doubling time (35). Especially, when the lesions are >10 cm, the preferred route of treatment is partial nephrectomy or selective arterial embolisation (16, 17). Massive retroperitoneal haemorrhage due to AML, also known as Wunderlich's Syndrome, has been found in up to 10% of patients (36). Although many angiomyolipomas do not show growth over time, those that occur with TSC are more likely to show progressive evolution. Conservative observation and follow-up examinations of asymptomatic patients with tuberous sclerosis and angiomyolipoma are recommended with bi-annual or annual imaging: US, CT, or MR imaging.

Selective arterial embolisation has been effective in the treatment of acute haemorrhage, with or without later surgery, or as initial treatment of angiomyolipoma (37-39). Although arterial embolisation is minimally invasive, it does not preserve renal function. It only has a temporary effect, it requires close clinical observation because of associated

complications, and, as a rule, it is ineffective when used alone. Regarding surgical treatment, a tumourectomy, partial nephrectomy, or total nephrectomy may be done. The surgical treatment that preserves the largest amount of renal tissue is tumour enucleation, which has been performed with excellent results, even for large angiomyolipomas (>20 cm). Tumour enucleation is practically applicable for patients with tuberous sclerosis who present with multiple and bilateral lesions (39-41). Total nephrectomy should be used very rarely; it is only justified in cases of uncontrollable bleeding, when there is risk to the patient's life, in central tumours, in the presence of extensive necrosis, when there is inflammation of the renal tissue, or when there is a diagnosis of renal carcinoma in the same kidney. Recently, cryotherapy has been suggested as a therapeutic option and may be associated with laparoscopy (42). The primary treatment objective of angiomyolipoma is the preservation of renal function, principally in those cases in which it is associated with tuberous sclerosis and the lesions are generally larger, multiple, bilateral, and recurring. Amongst the therapeutic options, tumour enucleation increases the potential for preservation of the renal tissue, followed by selective arterial embolisation and cryotherapy. Embolisation primarily controls the bleeding in the acute phase but may lead to greater loss of the renal function.

In conclusion, the basis of management for angiomyolipoma is to preserve of renal tissue and function, which can be effectively achieved with nephron-sparing surgical procedures such as tumour enucleation. However in some circumstances, it is necessary to do selective angioembolisation, partial nephrectomy, or even total nephrectomy. Especially in patients with tuberous sclerosis with large bilateral and multiple tumours, the aim of treatment is to preserve the greatest amount of efficient renal function.

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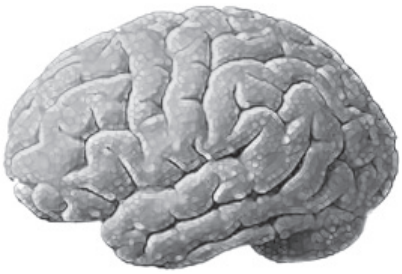

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