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*"The young and the old of widely different races, both with man and animals, express the same state of mind by the same movements."*
Charles Darwin

# THE BENEFITS OF USING NEW TOOLS FOR BEHAVIORAL INVESTIGATIONS IN ANIMAL EXPERIMENTAL MODELS

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[*]Dragica Selakovic and Jovana Joksimovic contributed equally (50% each) to this work and both should be considered first authors

# PREDNOSTI KORIŠĆENJA NOVIH PRISTUPA U BIHEVIORALNIM ISTRAŽIVANJIMA NA ANIMALNIM EKSPERIMENTALNIM MODELIMA

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## ABSTRACT

*The animal experimental models of emotional disorders attempt to reproduce features of human psychiatric disorders in laboratory animals by correlating the physiological and behavioral changes associated with specific emotional states, the etiology of disorders, and responses on drug treatments. Animal experimental models that accurately recapitulate clinical pathology are indispensable for understanding molecular mechanisms and advancing preclinical studies. Behavioral tests on animal models have been developed over time in two directions: to enable the acquisition of as many valid behavior data as possible, and constructing experimental models and procedures that represent a parallel with certain conditions in humans. In this review we discuss more information for the new tools in behavioral investigations in animal experimental models. Here we described evoked beam-walking (EBW) test as a new test for estimation of anxiety levels. The reliability of that test was confirmed in our studies by using nandrolone decanoate (ND) and testosterone enanthate (TE) in supraphysiological doses. Also, we defined a new approach to estimation of exploratory activity by using these tests and an improvement of detectability in standard evaluation of depressive state levels. Taking into account that behavioral investigation in animal models still has to remain indispensable in conducting of preclinical studies, we assume that new tools that can be applied in this field may improve the quality of research.*

**Keywords**: *behavior, anxiety, depression, animal experimental models.*

## SAŽETAK

*Primena animalnih eksperimentalnih modela u ispitivanju emocionalnih poremećaja na laboratorijskim životinjama predstavlja pokušaj reprodukcije karakteristika psihijatrijskih poremećaja u humanoj populaciji putem korelacije fizioloških i bihevioralnih promena udruženih sa specifičnim emotivnim stanjem, etiologijom poremećaja, i odgovorom na medikamentoznu terapiju. Animalni eksperimentalni modeli koji tačno reprodukuju kliničku patologiju su nezamenljivi za razumevanje molekulskih mehanizama i unapređenje pretkliničkih studija. Tokom vremena, bihevioralni testovi na animalnim modelima su se razvijali u dva pravca: da omoguće dobijanje što većeg broja validnih podataka o ponašanju, i za konstruisanje eksperimentalnih modela i procedura koje predtavljaju paralelu sa određenim stanjima kod ljudi. U ovom radu mi dajemo informacije o novim tehnikama u bihevioralnim istraživanjima na animalnim eksperimentalnim modelima. Ovde smo opisali test hodanja gredom sa evocirajućim stimulusom, kao nov test za procenu stepena anksioznosti. Pouzdanost ovih testova je potvrđena u našim prethodnim istraživanjima korišćenjem nandrolon dekanoata i testosteron enantata u suprafiziološkim dozama. Takođe, primenom ovih testova, definisali smo novi pristup pri proceni eksplorativne aktivnosti i pobolj-šanje detekcije pri standardnoj evaluaciji nivoa depresivnog stanja. Uzimajući u obzir da su bihevioralna istraživanja na animalnim modelima još uvek nezamenljiva u izvođenju pretkliničkih studija, pretpostavljamo da će nove tehnike koje mogu biti primenjene u ovoj oblasti poboljšati kvalitet istih.*

**Ključne reči:** *ponašanje, anksioznost, depresija, animalni eksperimentalni modeli.*

## ABBREVIATIONS

**EBW** - evoked beam-walking
**EPM** - elevated plus maze
**ND** - nandrolone decanoate
**OF** - open field
**TE** - testosterone enanthate
**TEA** - total exploratory activity
**TST** - tail suspension test

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## BACKGROUND

Ever since the first evidences for behavioral investigations in animal experimental models in 20th century, numerous behavioral tests have been developed (1). The animal experimental models of emotional disorders attempt to reproduce features of human psychiatric disorders in laboratory animals by correlating the physiological and behavioral changes associated with specific emotional states (face validity), the etiology of disorders (construct validity), and responses on drug treatments (predictive validity) (2). Primarily, behavioral tests have been developed to verify and support a theory of cognition and emotion. Also, it can be used to verify a psychopathology of mental health disorder (1). Development, improvement and increase in the number and reliability of behavioral test on animal models, led to the systematization of these studies (2). The result of this is a declarative categorization of preclinical studies into the procedures for examination of new drugs (2). Animal experimental models that accurately recapitulate clinical pathology are indispensable for understanding molecular mechanisms and advancing preclinical studies (3). Taking into account ethical consideration, certain drugs with potentially harmful effect on central nervous system can only be examined *in vivo* on experimental animals. Neurotoxicity of high dose of cisplatin, an anticancer drug, is just one of the examples (4).

Behavioral tests on animal models have been developed over time in two directions: to enable the acquisition of as many valid behavior data as possible (performed in standard maze), and constructing experimental models and procedures that represent a parallel with certain conditions in humans (experimental models of diseases) (1, 5).

Standard battery of behavioral tests include tests for evaluation of the emotional state (anxiety and depression) such as open field test (OF), elevated plus maze test (EPM), elevated zero maze test, tail suspension test (TST), forced swim test, evoked beam walking test (EBW), hot plate test, and tests for evaluation of other conditions such as spatial learning and memory impairment (Barnes maze, Morris water maze), and locomotion (rotarod test, grip wire test, beam-walking test). The majority of studies used classic tests of animal anxiety and depression such as OF and EPM (6). Since 1950-70s the open field test has been used to detect anxiety-like behavior in animals (7). The apparatus for OF test consists of a square arena (60x60x30 cm) made of black wood. During the trial the examiner is not present in the testing room. At the beginning of test each animal is placed in the centre of arena. The following parameters are scored: total distance moved, velocity, cumulative duration in central zone, frequency in central zone. Cumulative duration in central zone and frequency in central zone are considered as indicators of anxiolytic-like effect (6). EPM is used since 1986, for evaluation of anxious-like behavior and emotional reactivity of animals by means of a conflict between secure parts of the maze (two enclosed arms) and aversive parts of the maze (two open arms). EPM for rats consist of two open (50x20 cm) and two enclosed arms (50x20x30 cm) and an open roof with entire maze elevated 100 cm from the floor. Each rat is placed in the center of EPM with head facing toward the open arm and is allowed 5 minutes for free exploration. The following parameters are scored: cumulative duration in open arms, the number of entries into the open arms, total distance moved, and velocity. Number of entries and cumulative duration in closed arms are considered as indicators of anxiogenic effect (1). Various animal models of depression were developed and used in depression studies (8). Forced swim test (9) and tail suspension test (TST) are popular for screening antidepressant drugs (8). The basic principle of both tests that are designed for rodents is the quantification of immobility which is usually considered as a measure of ″behavioral despair″. The immobility is manifested as withdrawal from further escape attempts from awkward situation in which the animal was unwillingly placed. Instead of fear of drowning, the main motif for attempts to escape in forced swim test, in TST the animal is positioned upside down, facing the floor. That inconvenient position immediately initiates typical reaction by means of manifested tendency to climb and get upright in order to get normal upward body position. In this test, also, sooner or later, the animal is giving up from inefficacious attempts to establish its natural position, which is manifested as the state of immobility. Not surprisingly, all parameters obtained by means of TST represent different characteristics of immobility state - latency to the first immobility, the number of episodes of immobility, average duration of an immobility episode, total duration of immobility (10).

In order to improve the translational utility of animal models, the preclinical studies are designed in parallel to clinical strategy (2). Chronic stress has been widely used as a model of depression (11). Animal models of Alzheimer's disease with related comorbidities such as anxiety, depression, motivation and sleep disturbances are well used in behavioral studies (3). Lesion-based animal model appear to be relevant tools for studying the pathophysiology of the non-motor symptoms, such as apathy, anhedonia, depression, anxiety, symptoms of Parkinson's disease (12). The discovery of *disrupted-in-schizophrenia 1* gene indicated its crucial role in neurodevelopment. *Knock-out* mice for that gene, coupled with various environmental stressors, have been proven successful as models of schizophrenia (13). The phencyclidine animal model of schizophrenia has shown important role of cannabinoid system in pathogenesis of this disorder (14). The vast majority of pharmacological, environmental and genetic animal models have been used over years to study bipolar mania disorder (15). The genetic animal model of autism spectrum disorder has shown that subchronic administration of oxytocin could have therapeutic prosocial efficiency (16, 17).

The aim of this article was to allow more information for the new tools in behavioral investigations in animal experimental models, established in Laboratory for behavioral investigations at Faculty of Medical Sciences, University of Kragujevac. The article represents an overview of some new tests, innovative analyzes and improvements achieved in sensitivity of the standard tests.

### 1. Introduction of new tests for estimation of anxiety levels

#### 1.1. Evoked beam-walking test

In order to allow better insight to the way how certain mood disorders can influence physical performance we investigated the connection between those two behavioral patterns in the apparatus constructed for standard beam-walking test (18). Although this test was originally designed for estimation of motor coordination, integration (19), balance performance (20) and motor skills, we noticed that the main stimuli for the animal movement in this maze was to avoid narrow and unstable (unsafe) space (40-50 cm above the floor) that animal was placed on. All mentioned characteristics of the maze represent sufficient stimuli that forced the experimental animal to look for the safe place that could be found only in the goal (safe) box, on the opposite side of the maze (Figure 1). We concluded that interconnection between inconveniences caused by specific environment and consequent motor response (escape reaction) could be a good ground for testing alterations in that specific motor response induced by the changes in provoking stimuli. The basic idea was that increased anxiety levels would induce more prompt reaction and result in further decrease of time to cross the beam that was previously recorded in standard beam-walking test. We hypothesized that the level of decrease in time to cross the beam (comparing to previously achieved time in standard beam-walking test) should be proportional to anxiety levels

since the only alteration comparing to standard beam-walking test supposed to be introducing of new anxiety-provoking stimuli (during beam-walking test) that is strong enough to trigger the fleeing reaction of the animals and to potentiate anxiety reaction (inappropriate) to the intensity of additional stimuli.

Figure 1. The apparatus for the evoked beam-walking test.

Therefore, immediately after completing the standard beam-walking test on pretrained rats, at the same apparatus, each rat was placed at the end of the beam opposite to the goal box, while the experimenter started tapping (every 3 seconds) with a metal stick at the base of the stainless steel pole while rat traversed the beam (anxiety provoking pattern). The tapping was performed until the rat reached the goal box. The velocity (relative to the previously performed beam-walking test) was used as an indicator of anxiety. The beam-walking test was conducted under proper illumination. The results obtained in the study where the evoked beam-walking test was initially applied (18) estimated behavioral effects of food restriction. In accordance to values for parameters that were previously verified as the indicators of anxiety, the results obtained in the evoked beam-walking test also showed anxiolytic-like behavior in the rats with total food restriction, indicating that this test could also be suitable for estimation of anxiety levels. Velocity decrement in EBW test was, at least partially, attributed to food-seeking behavior in the tested group of animals.

The reliability of EBW test was confirmed in another study (21) where we investigated behavioral effects of nandrolone decanoate (ND), an anabolic androgenic steroid that is often misused among athletes and non-athletes. As shown in Figure 3, anxiogenic-like pattern of behavior following four weeks of treatment with ND in supraphysiological dose (in order to mimic heavy human abuse) was clearly observed in several ordinary behavioral tests, such as open field test (decrease in total time spent in centre zone, and elevated plus maze test (decreased cumulative duration in open arms), but also in EBW test. Decreased time to cross the beam in the EBW test, as an indicator of increased anxiety following chronic ND administration, fulfilled the spectrum of behavioral alterations in that study.

We also used this test in the study where we evaluated the opposite effects of chronic treatment with ND and exercise on anxiety levels in rats by analyzing the possible involvement of alterations in hippocampal parvalbumin-positive interneurons (22). Since the results of that study confirmed the role of hippocampal GABAergic content in mediation of both anxiogenic effect of anabolic androgenic steroids and anxiolytic effect of exercise, it was good opportunity to estimate the possible connections of parameters obtained in EBW test with the number of PV immunoreactive neurons in specified hippocampal regions. As shown in Figure 2, the usefulness of EBW test was confirmed by means of correlation between percentage of shortening time to cross the beam and the number of PV immunoreactive neurons in CA1 region and dentate gyrus of hippocampus. Furthermore, the validity of this test was potentiated by the fact that the correlation between the parameter obtained in EBW test and the number of hippocampal parvalbumin positive interneurons was even better compared to the correlation observed with the basic indicator of anxiety level (cumulative duration in open arms of EPM) in hippocampal dentate gyrus (Pearson's r = 0.50, p = 0.004 vs. r = 0.45, p = 0.01).

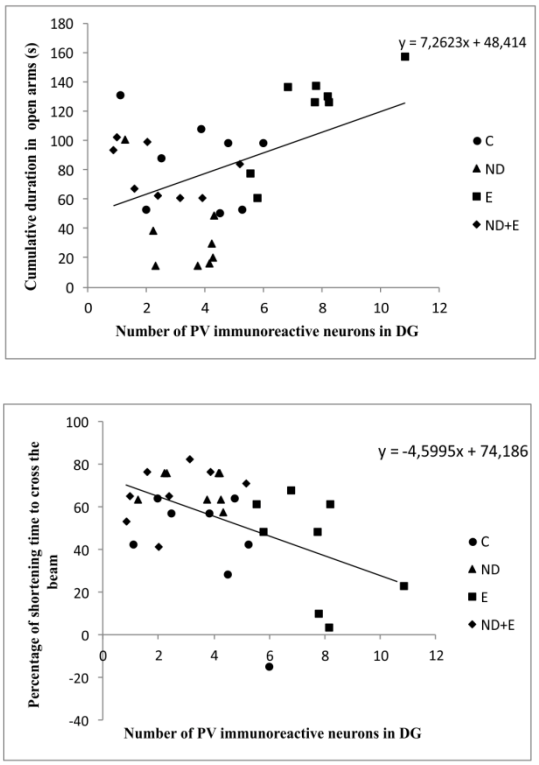


Figure 2.* Relationship between the number of PV immunoreactive neurons in dentate gyrus (DG) of hippocampus and the cumulative duration in open arms (left) and the shortening the time to cross the beam in the EBW test (right).

Simple regression analysis indicated that the number of PV neurons in DG was positively correlated with cumulative duration in open arms (F = 7.481, Pearson's r = 0.45, p = 0.01) and strongly negatively correlated with the shortening of time to cross the beam (F = 9.621, Pearson's r = 0.50, p = 0.004).

*Results previously published in PLOS ONE (12(12): e0189595), edited with permission of corresponding author.

### 1.2. The use of the tail suspension test for evaluation of anxiety state levels

In the same study (21), we proposed that results obtained in the tail suspension test could be used as parameters not only for evaluation of depression, but also as potential indicators for estimation of anxiety state levels. TST is considered as one of the most commonly used test in behavioral studies for evaluation of depression and estimation of various therapeutic approaches for depression treatment. Still, there are a few reports suggesting that this kind of test could be used in order

to assess different mood disorders, such as anxiety (23, 24). The basic idea that we tested in that study was funded on the assumption that parameters used for evaluation of depressive state levels (and also for antidepressant effects of drugs) could be used, on the same ground, for testing the anxiety related patterns in behavior by means of quantification of the efforts that animals perform when facing both new and irritating situation (like tail suspension test implicates). Since antidepressant effects may be accompanied with anxiety-like behavior (25-27), we proposed that increase in latency to the first immobility (the time needed to give up in attempts to escape), decrease in the number of episodes of immobility (the number of attempts to escape), and decrease in the total duration of immobility (total time with no attempts to escape) could be considered as indicators of higher level of anxiety. The chronic treatment with supraphysiological dose of ND significantly increased the latency to the first immobility and, also, significantly decreased the total duration of immobility when compared to the control group, even with no significant change in the number of episodes of immobility. In conclusion of that study, we proposed those results as a potential (indirect) evidence of anxiety-like behavior after chronic ND treatment. The proposed conclusion is in accordance with the results of all previous tests initially designed for evaluation of anxiety state levels that were performed in that trial.

**2. New approach to estimation of the exploratory activity**

Since the exploratory activity observed in the elevated plus maze test can be directly affected by changes in anxiety (28, 29) in the manner that decreased exploratory activity can be considered as the manifestation of increased anxiety levels, we focused on the quality of the exploratory activity estimation. Also, we noticed in numerous studies that the number of head-dippings was the only parameter used for the estimation of the exploratory activity (i.e. anxiety) in EPM test. However, analyzing video material obtained in our previous behavioral investigations, we noticed the fact that the exploratory activity of the animals during EPM test does not occurs only in the open arms of the maze (as head-dippings), but also in the closed arms. The behavioral pattern of exploratory activity that takes place in the closed arms is the rearing, well known and widely accepted as the parameter for the exploratory activity in OF test. Even more, the rearing behavior could also be observed in the open arms. Therefore, we decided to count all behavioral patterns for exploratory activity and defined it as the number of rearings, the number of head-dippings and total exploratory activity (TEA), expressed as the number of TEA episodes. We calculated the number of TEA episodes as the sum of patterns of exploratory activity observed in both the closed arms (the number of rearings) and in the open arms (the number of head-dippings and rearings, the behavioral pattern that can, although rarely, be observed in the open arms, as well), representing the overall exploratory activity in EPM.

Such a new approach for estimation of the exploratory activity (i.e. anxiety) in EPM test we applied in the study (30) that evaluated the behavioral effects of prolonged exercise (six weeks swimming protocol) and chronic testosterone enanthate (TE) administration in EPM test. This study also included the combined effects of those two simultaneously applied protocols (combined group). At the same time, we also followed the total duration in open arms, since it is considered as the key indicator for anxiety state levels alterations induced by any applied protocol, according to criteria for results obtained in the EPM test. Prolonged exercise protocol induced significant increase in cumulative duration in open arms of EPM, while chronic TE treatment also resulted in significant decrease in time spent in open arms comparing to all groups. The anxiogenic effect of TE was also observed in the combined group. Similar effects of two applied protocols were manifested by the number of rearings (closed arms). The swimming protocol induced significant increase in the number of rearings compared to all groups. On the other hand, supraphysiological dose of TE produced significant decrease of rearing compared to the control and exercise groups. The number of head-dippings also varied depending on the applied protocol. While TE administration significantly decreased this pattern of exploratory activity comparing to all investigated groups, the swimming protocol resulted in a significant increase in the number of head-dippings when compared to both TE and the control groups. The number of TEA episodes, as a new parameter for overall exploratory activity in EPM, the most clearly confirmed the opposite effects of applied protocols on alteration in anxiety level. So, the number of TEA episodes was significantly increased in the exercise group, while chronic treatment with TE resulted in a significant decrease compared to all groups. Furthermore, simultaneous administration of TE, along with swimming training, significantly decreased anxiolytic effect observed in exercise group by means of the number of TEA episodes.

Validity of this approach in analysis of the exploratory activity was confirmed by simple regression analysis that revealed that both the number of rearings and the number of TEA episodes (Figure 3) were significantly positively correlated with the cumulative duration in open arms (Pearson's $r=0.60$, $p<0.001$, $r=0.70$, $p<0.000001$, respectively), that is usually considered as a crucial indicator of anxiety state level in EPM. As shown in Figure 4, the correlation between the exploratory activity observed in inner parts of EPM (closed arms), expressed the number of rearings, and the total exploratory activity in EPM was strong and, expectedly positive (with the extremely high significance – $p = 2.20E^{-10}$).

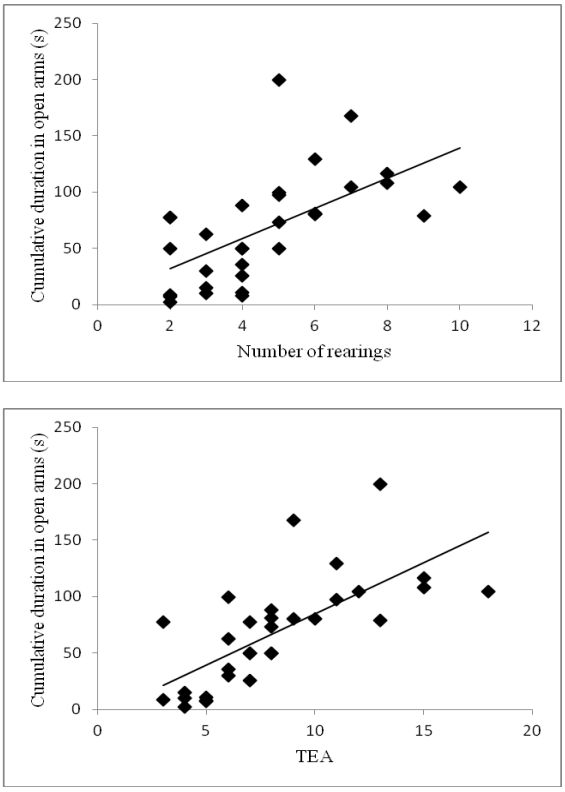


Figure 3.* Relationship between the number of rearings (left) and the total number of exploratory episodes (right) and the cumulative duration in open arms. Simple regression analysis indicated that the number of rearings, as well as, the total number of exploratory episodes, was positively correlated with cumulative duration in open arms (Pearson's $r = 0.60$, $p<0.001$ and $r = 0.70$, $p<0.000001$, respectively).

*Results previously published in Neuro Endocrinol Lett. 2016 Oct;37(5):383-388, edited with permission of corresponding author.

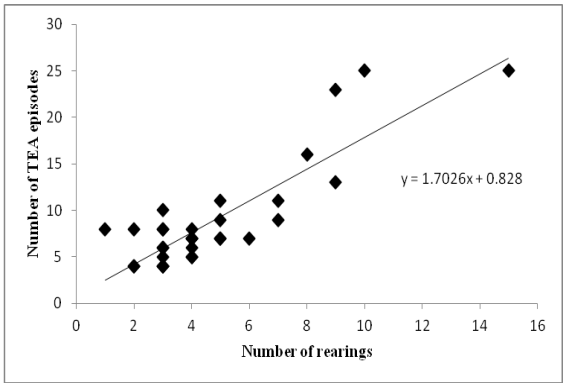


Figure 4.

In one of the mentioned study (testing the alterations of hippocampal PV interneurons induced by chronic treatment with ND and exercise) we, also, wanted to highlight importance of the exploratory activity in all parts of EPM. As shown in Figure 5, the exploratory activity that occurred in closed arms (expressed as the number of rearings), that is usually ignored in standard analysis, was observed in all experimental groups. The contribution of the exploratory activity in the closed arms in the total activity was significant in all experimental groups, with very small variations between the groups. The average contribution of the number of rearing in total exploratory activity in this study was 54.37±3.09, confirming that previous approach to exploratory activity analysis in EPM, focused on the behavioral patterns in open arms, underestimated the significant contribution of the exploratory activity in the closed arms.

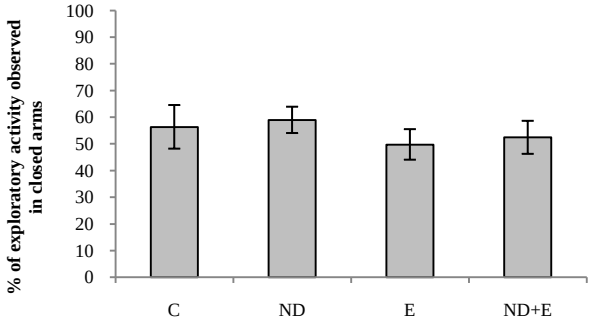


Figure 5.

## 3. Improvement of detectability in standard evaluation of depressive state levels

### Average duration of an immobility episode

Tail suspension test is based on the assumption that an animal will actively try to escape from an inescapable but moderately stressful situation (31). Rats are suspended by the tail attached to the adhesive tape, so that their bodies dangled in the air facing downward, both acoustically and visually isolated. Immobility is considered as a state of the animal with no visible voluntary movement (less than 1 cm) of head, body or limbs for 5 seconds or more. Involuntary swinging is considered as immobility. The test lasts for six minutes. As shown in Figure 6, the apparatus for TST consists of metal frame (60 x 60 cm) and circular barrier (25 cm in diameter) with the central opening (1.5 cm in diameter) where the tails were slipped through, 1 cm bellow the position of the adhesive tape on the tail, in order to prevent tail climbing. The impact of the barrier usage may be considered as substantial intervention, since the tail climbing represents one of the basic limitations on the use of TST in rodents (33). Behavior of the rats is usually recorded by a video camera, and records are then analyzed in order to determine the following standard parameters: the latency to the first immobility, the number of immobility episodes, and the total duration of immobility. However, analyzing numerous experiments obtained in TST, we noticed intriguing circumstances in which none of standard parameters obtained in TST could allow statistical confirmation for depressive state level alterations. Indeed, observed under specific conditions that statistical analyzes of neither the number of immobility episodes nor the total duration of immobility could express significant difference although clear pro and/or antidepressant effect was obvious. In order to overcome that problem we introduced an average duration of an immobility episode as the new parameter for TST. The average duration of an immobility episode was calculated as the ratio of total duration of immobility and the number of immobility episodes (10).

Figure 6.

We presented the usage of this parameter for the first time in the study where we evaluated the alterations of the oxidative status in rat hippocampus and prodepressant effect of chronic testosterone enanthate administration (32). The chronic (6 weeks) testosterone TE treatment in supraphysiological dose produced prodepressant effect in TST parameters, by means of parameters obtained in TST. On the other hand, prolonged exercise protocol (swimming for 1 h/day, 5 days in a row with 2 days break) induced the opposite effect. Interestingly, the newly proposed parameter, the average duration of an immobility episode, expressed more sensitivity for detection of alterations in depressive state when compared to standard TST parameters - the latency to the first immobility and the number of immobility episodes, and even to the total duration of immobility, that is considered as the main indicator for depressive behavior in TST. Confirmation for the usefulness of this parameter was obtained in this study by the fact that its values significantly correlated with the levels of oxidative stress markers, such as index of lipid peroxidation expressed as thiobarbituric acid reactive substance, and superoxide dismutase activity.

We also included this parameter for TST in the following study that evaluated the role of neuropeptide-Y in nandrolone decanoate-induced attenuation of antidepressant effect of exercise (10). Our results showed that prolonged exercise protocol induced antidepressant effect in TST, while chronic nandrolone decanoate treatment attenuated this beneficial antidepressant effect of exercise as measured by TST parameters.

Two standard TST parameters, the latency to the first immobility and the number of immobility episodes, failed to confirm statistically the alterations in depressive state levels. At the same time, the average duration of an immobility episode, as well as the total duration of immobility, was able to detect alteration in this behavioral pattern.

**Expert opinion**

Taking into account that behavioral investigation in animal models still has to remain indispensable in conducting of preclinical studies, we assume that new tools that can be applied in this field may improve the quality of research. Therefore, we suppose that our contribution in establishing of the new tests, parameters and alternative analyzes in behavioral research may be motivational for the new improvements in this field.

## ACKNOWLEDGEMENTS

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# PSYCHOLOGICAL FUNCTIONING OF ADOLESCENTS FROM VIOLENT FAMILIES

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# PSIHOLOŠKO FUNKCIONISANJE ADOLESCENATA IZ PORODICA SA NASILJEM

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## ABSTRACT

***Objective.*** *The aim of the study was to determine the association of family violence with the functioning of the family system, psychological functioning and development of specific characteristics of the adolescents who grew up in a violent families.* ***Methods.*** *The study was carried out on a sample of 308 adolescents, aged 15–18, divided in a FV group of adolescents coming from dysfunctional families (n=68) adolescents who grew up in violent families and were exposed to family violence, which was reported and processed, and a control group (n=240) adolescents from families in which according to the CPRS-R Questionnaire there was not found any form of violence, alcoholism or any other psychosocial pathology. FACES III Scale and Questionnaire for the examination of psychosocial characteristics of the adolescents and family (Revised CPRS-R) were used for measuring dimensions of family functioning, Cybernetic model of personality dimensions (CON-6) for conative functioning of the adolescents, Test for reasoning images (TRI) for measuring intellectual development and the Scale of Latent Maturity (SLM) for determining the level of psychological maturity. The data were processed by using linear regression analysis.* ***Results.*** *Family functioning disorders caused by family violence were significantly and highly associated with psychological functioning of the adolescents: concern about family relationships (β =-.968), need for professional help (β =-.873), cooperation with people (β =.523), orientation towards the future (β =.669), latent maturity (β =.618), psychosomatic reactions (β =-.509), dissociative reactions (β =-.591), excused absence from school (β =.618), unexcused absence from school (β =-.824), memory disorders (β =-.541), night fears (β =-722), running away from home (β =-.569), breaking rules and getting punished at school (β =-.569), conflicts with peers (β =-.460). Conative functioning of the adolescents exposed to a violent family environment showed a pathological form of functioning in dissociative, anxiety reactions and social adaptability.* ***Conclusion.*** *Exposure of adolescents to a violent family environment was associated with changes in psychological functioning, development of specific personality characteristics and risk of developing internalized and externalized symptoms when it came to social-adaptation, anxiety and dissociative reactions. The mediators between family violence and psychological functioning and development of the adolescents was family cohesion and disorders within the marital dyad.*

***Keywords****: family violence, psychological functioning of the adolescents, specific psychological features of adolescents.*

## SAŽETAK

***Uvod/Cilj.*** *Cilj istraživanja je utvrđivanje povezanosti porodičnog nasilja sa psihičkim funkcionisanjem i razvojem specifičnih osobina adolescenata.* ***Metode.*** *Istraživanje je sprovedeno na uzorku od 308 adolescenata, uzrasta od 15-18 godina, podeljenih u PN grupu adolescenata (n=68), koji su odrastali u porodicama sa porodičnim nasiljem i izloženi nasilju, koje je prijavljeno i procesuirano, i kontrolnu grupu (n=240) adolescenata iz porodica u kojima Upitnikom CPRS-R nije utvrđen bilo koji oblik nasilja ili druga psihosocijalna patologija. Skala Faces III i Upitnik za ispitivanje psihosocijalnih karakteristika mladih i porodice (Revidrani CPRS-R) korišćeni su za merenje dimenzija porodičnog funkcionisanja, Kibernetički model dimenzija ličnosti (KON-6) za konativno funkcionisanje adolescenata, Test rezonovanja likova (TRL) za merenje intelektualnog razvoja i Skala latentne zrelosti (SLZ) za utvrđivanje razvoja psihičke zrelosti. Podaci su obrađeni linearnom regresivnom analizom.* ***Rezultati.*** *Poremećaji porodičnog funkcionisanja izazvani nasiljem značajno (p ≤0.01) su i visoko povezani sa psihičkim funkcionisanjem adolescenata: brigom za porodične odnose (β=-.968), potrebom za stručnom pomoći (β=-.873), saradnjom sa ljudima (β=.523), orijentacijom ka budućnosti (β=.669), latentnom zrelošću (β=.618), psihosomatskim reakcijama (β=-.509), disocijativnim reakcijama (β=.-591), opravdanim izostajanjem iz škole (β=.618), neopravdanim izostajanjem (β =-.824), poremećajima pamćenja (β=-.541), noćnim strahovima (β =-.722), bežanjem od kuće (β =-.569), kršenjem pravila i kažnjavanjem u školi (β =-.569) i sukobima sa vršnjacima (β =-.460).* ***Zaključak.*** *Izloženost adolescenata porodičnom nasilju povezano je sa promenama psihičkog funkcionisanja, razvojem specifičnih osobina ličnosti i rizikom od nastanka internalizovanih i eksternalizovanih simptoma u socijalno-adaptivnim, anksioznim i disocijativnim reakcijama. Medijatori između porodičnog nasilja i psihosocijalnog funkcionisanja i razvoja adolescenata su porodična kohezivnost i poremećaji u bračnoj dijadi.*

**Ključne reči:** *porodično nasilje, psihološko funkcionisanje adolescenata, specifične osobine adolescenata.*

## ABBREVIATIONS:

**FV –** Family violence
**PN –** Porodice sa nasiljem

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## INTRODUCTION

### Functioning of a violent family system

The study of De Fife et al. (1) pointed out to the relationship between family violence (FV), family functioning problems and numerous psychosocial developmental difficulties of adolescents. Violent family showed a pronounced dysfunction within the marital dyad, which was reflected on all family subsystems. Role reversals in a violent family were associated with failing to satisfy basic psychological needs for security, love, belonging and respect which hindered individual functioning of one or more family members, that is, subsystem differentiation (2).

Family violence was often the result of pronounced violent parents' mental health problems. The structure of a violent person was predominantly characterized by emotional dysregulation, hostility, impulsiveness, bipolar disorder, antisocial personality disorder, substance use disorder (3). Intimate partner violence was significantly associated with poor mother's mental health (4). Abused women suffered more from a high level of depression, anxiety, phobias, posttraumatic symptoms and low self-esteem; they had suicidal ideas and suffered from attempted suicide, auto destructive actions; alcohol, nicotine and other addiction; sleep and diet disorders; social functioning disorders; behavioral problems and unsafe sexual behavior (5). Poor mental health of the mother was associated with the child's increased aggressive behavior, lowered mother's warmth and more frequent physical and psychological abuse of the child. Negative consequences of increased aggression and deficit of the mother's mental health and warmth significantly affected the adolescent's social and behavioral development (6).

The most pronounced consequences of the violence family system were the changes in family dimensions of cohesion and communication among subsystems (7). Weakening of closeness and communication within the marital dyad brought about the weakening of emotional association between the mother and the children (7). Within violent families, 20-60% of children were themselves exposed to some form of violence – physical, psychological or sexual (8).

### Consequences of adolescent growing up in violent families

The consequences of growing up within violent families were emotional, cognitive, behavioral and health problems (9).Long-term exposure to negative traumatic stress on the part of adolescents from violent families was associated with the sensation of fear, tremor, hyperactive attention and responses that might bring about cognitive problems: low cognitive and school performance (10), lost or inhibited skills of problem and conflict management (11), accepting violent forms of behavior and attitudes, belief in rigid sex stereotypes and men's privileges (12). During their social development, adolescents exposed to family violence had difficulties in establishing contact with their peers (7). Growing up in violent families was associated with increased risk of PTSD (posttraumatic stress disorders) and alcohol addiction (13). Psycho-socio-pathology of adolescents growing up in violent families was associated the most with the characteristic of the families themselves (14). Close family association and social support were important for internalized symptoms: anxiety, acute state of fear, depression, hypersensitivity, acquired helplessness, posttraumatic stress disorder (PTSD), whereas low cohesion intensified externalized symptoms such as aggression, behavioral disorders (lying, thefts, fights, idleness), antisocial and delinquent behavior, substance use disorder, impulsive behavior, attention deficit disorder (ADD), attention deficit and hyperactivity disorder (ADHD), oppositional defiant disorder (ODD) (15).

### Symptoms of adolescent from violent families

Psychopathological symptoms of a child within a violent dysfunctional family system occurred as a consequence of the inability to reduce anxiety and family structure disorder. Resolution of a high fusion level within the family was a sign of low differentiation, because high tension and inter-dependence level brought about emotional cut-off, most frequently at the onset of the first symptoms. High family and marital anxiety was centered on the child/adolescent. The result was the very dysfunction of the child/adolescent (16).

Adolescents fromviolent families had more pronounced symptoms of posttraumatic stress disorders (17) and showed the development of posttraumatic symptomatology: depression, suicidality, anxiety, substance use disorder, delinquency and somatic difficulties (18). Adolescent exposure to verbal and physical abuse was accompanied by depression, delinquency and suicidal ideas (19). Pronounced externalized adolescent problems predicted a high degree of negative emotional expressiveness, hostile reactions and trans-generational transfer of violence (20).

The theoretical concept of this survey was represented by a system family theory, which, through an eclectic approach of theoretical systems, highlights the functioning of a family system in general and its subsystems in the form of establishing family homeostasis and morphogenesis or the ability to adapt to changes (21).

### Aims of survey

The basic aim of the survey was to determine the association between the functioning of a violent family system and the changes in psychological functioning of adolescents coming from such families.

Specific aims of the survey:

1. To determine the effect of family violence on intellectual functioning of adolescents coming from such families.
2. To determine the effect of family violence on conative functioning of adolescents coming from such families.
3. To determine the effect of family violence on the problems of psychosocial development and behavioral disorders.

4. To determine the effect of family violence on the level of pronunciation of psychological maturity dimensions on the part of adolescents coming from such families.

## METHODS

### Sample/Participant

The study was conducted on a sample of 308 adolescents, aged 15-18 (M=16.5 ± 1.5). There was no difference between the groups by the age (t (198) =-1.09, p=.125. The sample consisted of two subsamples: the FV one was made up of adolescents growing up in violence families (FV) and exposed to FV, which was reported to and processed at the Centre for Social Work in Vranje (n=68 / 22.8%, 52.9% males and 47.1% females) and the control one made up of (240 / 77.3% , 42.5% males and57.5% females) adolescents coming from families in which there had not been found any type of violence and social pathology using a Revised CPRS-R Questionnaire (22). FACES III Scale (23) and Questionnaire for the Examination of Psychosocial Characteristics of the Adolescents and Family (Revised CPRS-R)were used for measuring dimensions of family functioning, the Scale of Latent Maturity (SLM) (24) for determining the level of psychological maturity, Test for reasoning images (TRI) (25) for measuring intellectual development, Cybernetic model of personality dimensions (CON-6) (26) for conative functioning of the adolescents.

### Procedures

After being acquainted with the Approval by the Ethics Committee of the Medical Faculty in Kragujevac as regards conducting the design of study and upon getting the respective Consent by the Centre for Social Work in Vranje and Secondary School Gimnazija „Bora Stanković" in Vranje, the respondents gave, after being acquainted with the aims of the study, written information consent for participating in the study.

The testing of adolescents coming from functional families was conducted by a school psychologist in the school premises. The testingof adolescents coming from violent families was conducted by psychologists and specialist teachers, who did therapeutic work with the parents and the adolescent or the adolescent only in the Centre's premises. The study was anonymous. Testing total test material took one hour. KON-6 (Test conative functioning personality) respondents filled out for 30 minutes, while answering the Questionnaire (CPRS-R) and FACES III lasted up to 15 min. The study was conducted during the period from 2010. to 2012.

### Instruments

The results for the control group of adolescents coming from families without family violence was obtained by using the revised Questionnaire for determining psychological and social pathology of family members - Revised Conners Parent Rating Scale (CPRS-R). It contains 20 items (22). In this study, reliability estimates for the scores were α=.94.

Family dimensions cohesion and adaptability were measured by using the Scale of Family Adaptability and Cohesion (FACES III), which consisted of 20 items. Obtained results were expressed by using raw scores and categories (cohesion: low - dissociated, mild - separated, developed - coherent and high - intertwined; adaptability: low, moderate - flexible, developed - structured, high - rigid) (23) . In this study, reliability estimates for the scores were α =.89.

Conative dimensions, measured by the KON-6 Conative Personality Dimensions, were: reaction activity-introversion/extroversion, psychosomatic reactions, anxiety-defence reactions, aggressive-response attacks, dissociative reactions, integrative reaction-social adaptation. It contains 180 items. Conative personality dimensions were expressed in the following categories: superior, above-the-average, average, below-the-average, and pathological functioning (26). In this study, reliability estimates for the scores were α =.96.

Psychosocial development of latent maturity: accomplished level of forming personal, social identity and personality identity (differentiation of personality, inclusion into a social community, defining psychological and ideal I) was surveyed by using the Scale of Latent Maturity. The Scale of Latent Maturity was made up of five subscales, with a total ofmultiple choice questions which the respondents answered by choosing from five given anwers: I completely agree, I mostly agree, I cannot make up my mind, I mostly disagree, I do not agree at all. The subscales of latent maturity were: cooperation with other people, frustration tollerance, trust in people, selflessness and orientationtowards the future. As an answer to the question: What are my plans and desires for the future, the respondent should choose from one of the five given answers. Raw scores at these subscales were turned into categories (low, below the average, average, above the average, pronounced), on the basis of the tables in the manual. The values of these scales were accumulated and Latent maturity was obtained. According to the table, raw scores were defined as maturity categories: pronounced immaturity, predominantly immature behaviour, mature behaviour with occasional regression, predominantly mature behaviour, pronounced mature behaviour (24). The reliability of the Scale of Latent Maturity in this study was α=.92.

Overall intellectual ability was measured by using the Test of Reasoning Images (25). The test included 45 tasks with six possible answers. Raw scores were transfered into intelligence quotient (IQ), according to standardised tables, into five categories: below the average, average, somewhat above the average, high and gifted. The reliability of the Scale was α=.97.

The reliability was medium and high value α confirms the validity of applied techniques and number of measured dimensions.

**Statistical analysis**

Linear regression analysis was used for evaluating the contribution of independent variables to a certain outcome of psychological functioning of adolescents exposed to family violence.

Determining association between the respective types of family: functonal one and a violent family, and psychological functioning of adolescents coming from above mentionedrespectivefamilies, was performed by using linear regression analysis, where the predictor variable was: type of family with two categories coded as dummy variables: 0=functional and 1=dysfunctional affected by violence.

The level of pronunciation of family and psychological variables was shown by using statistical description techniques.

The level of statistical significance was less than 1% ($p < 0.01$) and 5% ($p < 0.05$). Data analysis was performed using the software package SPSS version 11.5.

## RESULTS

The results obtained by using the linear regression analysis showed that family violence was a significant (p=.000) predictor of disorders in the functioning of the family and psychosocial development of adolescents growing up in and exposed to family violence (Table 1, Figure 1).

Family violence was significantly associated with cohesion (β=.605), among family subsystems. Functional families obtained an average value for the dimension of cohesion (M=41.050 ± 4.314), which corresponded to category 4 or highly pronounced closeness among family members, as compared with average values in a violent family environment (M=29.0881 ± .616) in terms of one category lower (3) which corresponded to achieved cohesion. Family violence was significantly associated with un/employment of the father (β=.618, p=.000). In violent families, half of the entire numbers of fathers were unemployed (50.0%, vs. 1.3% in functional families). Family violence was significantly associated with fathers' mental health. There was a negative correlation (β=-.489) between mental problems of the father and family violence.Within violent families, 25.5% of fathers had a certain mental disorder, compared to 0.0% in functional families. Family violence significantly affected father – children relationship. There was a negative correlation (β=-.445) in nonviolent father's behavior towards the children. The father's punishing or being violent to the children was more frequent within violent families (17.6% vs. 1.3%), as compared with the father – children relationship within functional families.

Disorders in family functioning caused by family violence were (p=.000) highly associated with disorders in psychological functioning of adolescents in the form of pronounced being worried for family relationships. Obtained highly pronounced negative correlation (β=-.968) and a high level of children being worried about their family relationships (100% vs. 0.4%), were associated with the need for psychological and psychiatric help with the aim of restoring disrupted psychological balance (β=-.8739). Obtained negative correlation between family violence and internalized adolescent homeostasis growing up in a violent family environment showed that 88.2% got or is still getting expert help from psychologists and psychiatrics, compared to 1.7% adolescents from functional families.

Adolescents from violent families were significantly associated with impaired intellectual development (β=.357). Adolescents from functional families had a category of overall intelligence somewhat above the average (M=112.775 ± 7.307), as compared to adolescents from a violent family environment who showed lower or average intelligence levels M =104.235 ± 8.038).

Significant negative correlations were obtained between family violence and cognitive functioning. Adolescents from a violent family environment had bigger cognitive problems: memory disorders (β=-.541; 58.8% vs. 5.4%), attention deficit disorders (β=-.338; 76.5% vs. 27.9%), unexcused (β =-.824; M=15.00 ± 7.596) and excused leave from school (β =.618; M=51.39 ± 31.218 vs. M=16.04 ± 11.516). Difficulties in intellectual development and learning problems were accompanied with lower school achievement (β=-.408; M=3.25 ± 1.456 vs. M=4.339 ± .826). Adolescents from violent families had on the average good achievement at school, as opposed to adolescents from functional families who accomplished very good achievement on the average.

Negative correlations were obtained between family violence and conative functioning of the adolescents.

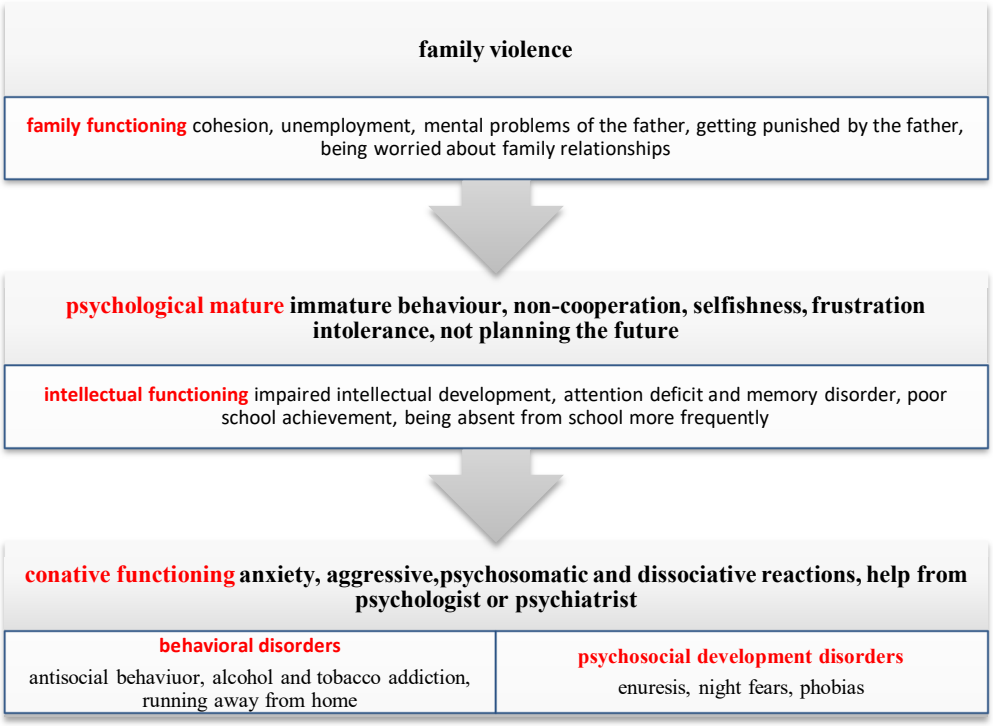
Adolescents from violent families had difficulties in conative functioning and ran the risk of developing psychopathological symptomatology due to increased psychosomatic (β=-.509;M=72.06 ± 26.058, category 4 – below the average functioning vs. M=47.05 ± 11.348, category 1- superior functioning), dissociative (β=.-591;M=70.68 ± 26.487, category 4 – below the average functioning, vs. M=41.92 ± 9.774, category 3 – average functioning), anxiety (β=-.393; M=94.44 ± 24.036, category 4 – below the average functioning, vs. M=69.91 ± 18.169, category 3 – average functioning) and aggressive reactions (β=-.359, M=99.97 ± 18.118, category 4 – below the average functioning vs. M=82.81 ± 12.214, category 3 – average functioning). Disorders in conative functioning were associated with impaired integrative and adaptive systems of adolescent socialization (β=-.455, M =77.47 ± 23.452, category 4 – below the average functioning vs. M=54.52 ± 13.257, category 3 – average functioning).

**Table 1.** Linear regression analysis of predictor variable– type of families:
0 - functional family, 1 –violent family

| Variable | R[a] | R [2b] | Adjusted R [2]**c** | B[d] | t –test [e] | P [f] |
|---|---|---|---|---|---|---|
| Latent maturity | .618 | .382 | .379 | .608 | 12.956 | .000** |
| Cooperation | .523 | .273 | .271 | .523 | 10.116 | .000** |
| Frustration tolerance | .416 | .173 | .170 | .416 | 7.543 | .000** |
| Trust | .368 | .135 | .132 | .368 | 6.527 | .000** |
| Selflessness | .348 | .121 | .118 | .348 | 6.120 | .000** |
| Orientation towards the future | .669 | .448 | .446 | .669 | 14.864 | .000** |
| Psychosomatic reactions | .509 | .259 | .257 | -.509 | -9.760 | .000** |
| Anxiety reactions | .393 | .155 | .152 | -.393 | -7.054 | .000** |
| Aggressive reactions | .398 | .159 | .156 | -.359 | -7.162 | .000** |
| Dissocative reactions | .591 | .349 | .346 | -.591 | -12.071 | .000** |
| Social adaptation | .455 | .207 | .204 | -.455 | -8.422 | .000** |
| Cohesion | .605 | .366 | .364 | .605 | 12.532 | .000** |
| Adaptability | .188 | .035 | .032 | .188 | 3.164 | .091 |
| IQ quotient | .357 | .127 | .124 | .357 | 6.298 | .000** |
| School achievement | .408 | .167 | .164 | .408 | 7.378 | .000** |
| Excused absence from school | .608 | .389 | .379 | -.618 | -11.360 | .000** |
| Unexcused absence from school | .824 | .678 | .677 | -.824 | -19.160 | .000** |
| Employed father | .618 | .381 | .379 | .618 | 12.902 | .000** |
| Punishing of the mother | .360 | .130 | .126 | -.360 | -6.365 | .000** |
| Getting punished by the father | .445 | .198 | .195 | -.445 | -8.189 | .000** |
| Marriage problems | .905 | .918 | .918 | -.958 | -55.313 | .000** |
| Mental problems of the father | .489 | .240 | .237 | -.489 | -9.222 | .000** |
| Alcohol abuse | .428 | .180 | .180 | -.428 | -7.806 | .000** |
| Attention deficit | .338 | .114 | .111 | -.338 | -5.924 | .000** |
| Memory disorder | .541 | .293 | .290 | -.541 | -10.608 | .000** |
| Night fears | .722 | .522 | .520 | -.722 | -17.222 | .000** |
| Enuresis | .323 | .105 | .101 | -.323 | -5.636 | .000** |
| Phobias | .586 | .343 | .340 | -.586 | -11.914 | .000** |
| Being worried about family relationships | .968 | .937 | .936 | -.968 | -63.376 | .000** |
| Conflict with peers | .460 | .212 | .209 | -.460 | -8.554 | .000** |
| Getting punished for breaking rules | .592 | .350 | .348 | -.592 | -12.100 | .000** |
| Running away from home | .569 | .325 | .321 | -.569 | -11.400 | .000** |
| Professional help | .873 | .762 | .762 | -.873 | -29.527 | .000** |

*Legend*:
[a] R-regression residual, [b] $R^2$ R square,
[c] Adjusted $R^2$ ,[d] β-quotient of correlation,[e] t –test, [f]p- significant p<0.01

**Figure1.** Effects of functioning family violence on adolescent psychological functioning: psychological maturity, conative, intellectual functioning, behavioral and psychosocial development disorders




Significant negative correlations were obtained between family violence and psychosocial development of adolescents.

The study showed that the adolescents growing up in violent families had significant development disorders in: night fears (β=.-722, 82.4% vs. 5.0%), phobias (β=-.586, 64.7% vs. 5.45%) and enuresis (β=-.323, 11.76% vs. .0%).

Obtained negative correlations pointed out that family violence significantly increased adolescent behavioral disorders: running away from home (β=-.569, 35.3% vs. .0%), breaking rules and getting punished at school (β=-.569, 44.1% vs. .8%), conflicts with peers (β=-.460, 29.4% vs. .8%), alcohol addiction (β=-.428, 79.4% vs. 37.5%).

Impaired emotional, intellectual and social development of adolescents from violence families were accompanied by slower development of psychological, latent maturity (β=.618, M=17.65 ± 2.215, category 4 – predominantly mature behavior, vs. M =12.00 ± 3.339, category 2– predominantly immature behavior) and maturity dimensions: cooperation with other people (β=.523, M=160.47 ± 16.279, category 4- high, vs. M=127.588 ± 25.958, category 2/3 borderline moderate/developed), orientation towards the future (β=.669, M=4.89 ± 0.466, category - pronounced need for accomplishing one's values in life, vs. M =3.088 ± .1.42, category – perceiving obstacles in planning the future and carrying out the potential), frustration tolerance (β=-.416, negative correlation pointed out to the fact that family violence lowered the readiness to deal with a failure; M=99.88 ± 10.225, category 4 - high, vs. M=83.382 ± 16.720, category 3 - developed), trust in people (β=-.368, negative correlation showed that family violence lowered trust in people, M=121.46 ± 12.368, category 3 - developed, vs. M=106.205 ± 13.143 , category 2 - moderate), lowered selflessness (β=.348, M= 71.21 ± 9.102, category 3- developed, vs. M=59.941 ± 15.233, category 2- moderate).

## DISCUSSION

Family violence significantly damaged functionality and structure of all the subsystems as well as their mutual association (27). Altered behavior consequently threatened to destroy the wholeness of a dysfunctional family, triggering chain changes in the functioning of other family subsystems and their mutual relationships in the form of intemperance, unpredictability, emotional cut-offs or distance, the feeling of failure to fulfill one's one role as well as the feeling of other people's failure to fulfill their roles (21).

Family violence exhibited pronounced unemployment and bad financial conditions. Family's failing to fulfill its financial role as regards providing necessary prerequisites for satisfying basic physiological and psychological needs was associated with failing to satisfy the motive for material and psychological security of the family's subsystems and bad emotional relationships within it (28). Disorders of emotional

relationships within dysfunctional families were reinforced by a significantly higher rate of children getting punished by parents in violent families. A large number of adolescents from violent families showed a markedly pronounced fear of the father (29). The quality of emotional relationships within violent families was damaged the most between the husband and wife i.e. within the marital dyad. The relationships within the marital dyad in violent families were characterized by the presence of various forms of violence and communication problems (30).

Structural and dynamic changes of violent families significantly affected the level of the adolescents being worried about their family relationships (31). Growing up of adolescents in violent families was significantly associated with difficulties in psychosocial functioning, because of which they showed an increased need for getting professional help from the psychologist and psychiatric with the aim of restoring homeostasis in their own system or attaining self-regulation and normal functionality of personality (32).

Violent families were significantly characterized by the presence of family pathology which disrupted the accomplishment of basic dimensions of cohesion and communication, lowering closeness, warmth and sense of unity (33).

Adolescent exposure to family violence made their intellectual development more difficult. Attention deficit disorder, adolescents' memory difficulties were associated with a significantly lower school achievement. Exposure to chronic negative family stress and various forms of violence affected increased excused and unexcused leave from school. A high rate of unexcused leave from school on the part of adolescents from dysfunctional families provided the basis for mandating educational-disciplinary measures. Obtained data on the significantly lower school achievement (11) of adolescents growing up in violent families represented the consequence of absence of cognitive stimuli provided in the family because of the following: fathers uninterested in carrying out their role as regards the in children's education, poor material conditions, pronounced psychopathology of the parents, low cohesion and impaired communication. Family violence brought about poor school achievement, due to overall decreased social and intellectual stimulation and lack of media influence: TV, the Internet, music, low physical activity, substance use disorder and emotional problems of adolescents (34). Certain studies of internalized problems of adolescents from violent families associated anxiety and depression with learning problems due to psychobiological mechanisms. High alert of the nervous system (arousal) and years' long exposure to stress led to decreased agility, learning problems and externalized symptoms (35).

Conative functioning of adolescents growing up in violent families was significantly different in hierarchically lower and higher dimensions (26). Adolescents from violent families showed significantly bigger depressive reactions (13, 36, 37), anxiety, aggressive, psychosomatic reactions and a higher risk for developing psychosomatic diseases (38). Disorders in the functioning of the Regulator of Defense Reactions led to the anxiety symptom, which provided the basis for pathological reactions such as: phobias, obsession, compulsiveness, sensory and emotional hypersensitivity, post-traumatic stress disorder (39, 40, 41). Characteristics of dysfunctional communication in violent families include lower levels of verbal expression and emotional reactions, lower tolerance to criticism and its intimate interpretation, which increased the anxiety and a number of reasons for violence (42). Reactions of the Attack Regulator or aggressive reactions were significantly more pronounced with the adolescents from violent families. Under unfavorable circumstances, increased aggressive reactions in this group might bring about a lot of externalized symptoms such as: impulsiveness, destructiveness, behavioral disorders, delinquency (43). Aggressive reactions of adolescents from violent families were associated with the mental health of the mother, lack of maternal love and warmth, physical punishing of the children (44).

Dysfunction of lower conative regulators on the part of the adolescents from violent families, psychosomatic, anxiety and aggressive reactions, was associated with significant disorders in the functioning of the supervising System for Regulating Function Coordination (45). Pathological values of the System for Regulating Function Coordination brought about the disorganization of cognitive and conative processes, motoric function disorders, schizophrenic, paranoid, manic, more severe forms of sensory and motoric conversion, some form of inhibitory conversion and fixated phobia, obsession and compulsion (46).

Disorders of hierarchically lower conative dimensions of adolescents from violent families brought about significant differences in the functioning of the highest Regulation Function Integration System. Pathological functioning of the Regulation Function Integration System was associated with social dis-adaptation, lower level of social maturity and externalized symptoms. Exposure to family dysfunctionality caused by family violence along with parental psychopathology and low self-regulation on the part of the adolescents brought about antisocial behavior, alcohol addiction, aggression, hostile reactions and trans generational violence transfer (9, 19, 43, 47).

Psychosocial development of adolescents from violent families was significantly associated with a number of disorders (48). Family violence within the family was associated the most with the occurrence of night dears, phobias and enuresis. Psychosocial development disorders of adolescents from violent families were the consequence of long-term exposure to negative family stress, open conflicts and traumatic experiences. Traumatic symptoms, flash episodes, impaired communication and lack of closeness with parents were associated with the occurrence of psychosocial development disorders (49).

Growing up of adolescents in violent families was associated with a larger number of behavioral disorders.

Adolescents from violent families showed a significantly higher rate of abandoning their families and attempting suicide; they broke more school and social rules, because of which they got punished more often (50). Disrupted parent-children relationships, low cohesion, emotional loneliness, insecurity in social skills caused overall inadequacy, running away from problems and reality by abandoning their own home or attempting suicide. Poor material wellbeing and tendency to exhibit uncontrollability in communication enhanced suicide rate of adolescents from violence families (51).

The most significant data on the effect of family violence on psychological functioning of adolescents were obtained in the domain of psychological maturity development. Adolescents from a violent family environment scored two category lower psychological maturity when compared with adolescents from functional families. Psychological functioning of adolescents from violent families reached the level of predominantly immature behavior, as opposed to adolescents from functional families who, in their functioning, reached predominantly mature behavior. Family violence hindered integrative personality abilities in terms of achieving one's personal identity, i.e. family functioning disorders resulted in having difficulties when it came to achieving homeostasis on the part of the adolescent, as a subsystem.

Family violence was associated with the formation of immature personality, the kind of immaturity that *Hrnjica* (24) called "underdeveloped personality immaturity“. The feeling of not being loved, absence of closeness with parents caused insecurity, emotional instability, identification and integration process disorders. This type of non-maturity had its existing forms in antisocial behavior (alcoholism, delinquency, loitering, psychopathic) and neurotic behavior (escape from reality).

Adolescents from violent families had significantly lower dimensions of maturity; frustration tolerance, orientation towards the future (52). Diminished cooperation with other people on the part of adolescents from violent families was the result of low personality integrity. Lower level of association among values, characteristics, self-image and motives diminished control over acquired differentiation and the number of contact with other people (24). Anger and negative emotions of adolescents from violent families caused distrust and poor cooperation with other people (53). Impaired emotional, conative and intellectual development of the adolescents from violent families, together with learning problems and lower school achievement and psychological maturity caused uninterestedness and fear of the future (54).

Family psychopathology and family conflicts caused a high risk of alcohol problems on the part of adolescents from violent families. Family violence disrupted the relationship among the siblings, enhanced aggression and led to compulsory alcohol abuse (52).

Growing up in violent families whose functionality was both structurally and dynamically impaired, significantly disturbed psychological functioning of an adolescent, as a family subsystem. Difficulties in intellectual, conative and behavioral functioning brought about the inability of achieving personal integrity and attaining psychological maturity. Family homeostasis disorders were associated with difficulties in achieving personal homeostasis on the part of the adolescent in the process of morphogenesis and they were accompanied by the risk of occurrence of internalized and externalized symptoms.

**Limitations**

The results of the study were based on the self-evaluation values of family and personal dimensions. Therefore, the conclusions were associated with the respondents' ability to describe their own as well as their family dimensions.

Although the study encompassed a large number of family and psychological variables, some variables were not included (psychological or psychiatric evaluation of parents from violent families, duration and severity of exposure to family violence, duration of medical attention after reporting intimate partner violence), which might be the subject matter of some other study.

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## CONCLUSIONS

The study pointed out that growing up in a dysfunctional family system, causedby violence, multiply damaged in terms of basic dimensions, cohesion and communication among subsystems was associated with cognitive, conative, social and emotional-behavioral development and that it structured specific characteristics and significantly predicted psychopathological symptomatology of adolescents from such families.

Inter–family violence exposure during adolescent development predicted the following: pronounced level of being worried about family relationships, night fears, enuresis, need for professional help, lower level of cooperation with other people, lowered orientation towards the future and lower level of latent maturity acquired, pronounced psychosomatic, anxiety, aggressive and dissociative reactions, more frequent absence from school, pronounced attention deficit and memory disorders, running away from home, breaking rules and getting punished at school, conflicts with peers, lower frustration tolerance, distrust of people, selfishness, social mal adaptation, impaired intellectual development and alcohol addiction. The mediators between the functioning of

a violent family system and adolescent personality psychosocial development were: mental problems of the father, disrupted relationships within the marital dyad, low cohesion and unemployment and psychopathology of the father.

Scientific significance of this work was shedding light on the mechanisms of specific adaptation on the part of adolescents from violent families to growing up in a dysfunctional family system, i.e. inter–family violence families. The possibility of developing psychopathological symptoms pointed out to the need of undertaking certain preventive, mental-hygienic measures in working with adolescents growing up in and exposed to family violence.

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# RISK FACTORS AND CHARACTERISTICS OF FALLS AMONG HOSPITALIZED STROKE PATIENTS

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# FAKTORI RIZIKA I KARAKTERISTIKE PADOVA KOD PACIJENATA HOSPITALIZOVANIH ZBOG MOŽDANOG UDARA

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## ABSTRACT

*Falls and fall-related injuries during hospitalization may cause serious problems and consequences for patients, their quality of life as well as increased healthcare expenses. The aim of the paper were to assess fall risks and identify risk factors, related to falls among stroke patients. This was a retrospective cohort study that included 217 neurological patients with acute stroke who have experienced fall during hospitalization. Morse Fall Scale was used to estimate a likelihood of falling for hospitalized patients. In total, 1.4% patients with acute stroke experienced a fall during hospitalization. According to the fall risk assessment, 77% of the patients presented a high risk for falls. Women, older respondents and those who were hospitalized for period longer than 22 days and who had higher levels of care, had higher values of Morse score. The most common risk factors for falls are: the presence of other medical diagnosis, the use of disability aids while walking, the use of intravenous therapy, disorientation in time and space, and the largest contribution to Morse score comes from using disability aids while walking and transferring patients. Greater risk of falling was observed in older neurological patients with ischemic type of stroke and weakness on the left side of the body, patients with longer hospitalization period and those with higher level of care.*

***Keywords**: falls, stroke patients, Morse fall scale, hospitalization, risk factors.*

## SAŽETAK

*Padovi i povrede povezane sa padom tokom hospitalizacije uzrokuju ozbiljne probleme i posledice po pacijente, njihov kvalitet života i povećanje troškova zdravstvene zaštite. Cilj ovog rada je bio da se proceni rizik od pada i identifikuju faktori rizika u vezi sa padom kod pacijenata koji su imali moždani udar. U pitanju je retrospektivna kohortna studija koja je obuhvatila 217 neuroloških pacijenata sa akutnim moždanim udarom koji su doživeli pad tokom hospitalizacije. Morseova skala je korišćena za procenu verovatnoće pada hospitalizovanih pacijenata. Ukupno, 1,4% pacijenata sa akutnim moždanim udarom je doživelo pad tokom hospitalizacije. Prema proceni rizika od pada, 77% pacijenata je pokazalo visok rizik od pada. Žene, stariji ispitanici i oni koji su hospitalizovani duže od 22 dana i na višim nivoima nege imaju više vrednosti Morseovog skora. Najčešći faktori rizika za padove su: prisustvo druge medicinske dijagnoze, upotreba pomagala prilikom hodanja, intravenska terapija, dezorijentacija u vremenu i prostoru, a najveći doprinos Morseovom skoru dolazi od korišćenja pomagala prilikom kretanja/premeštanja. Veći rizik od pada imaju stariji neurološki pacijenti sa ishemijskim tipom moždanog udara i slabosti na levoj strani tela, pacijenti sa dužom hospitalizacijom i oni sa hospitalizacijom na višim nivoima nege.*

***Ključne reči:** padovi, pacijenti sa moždanim udarom, Morseova skala, hospitalizacija, faktori rizika.*

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## INTRODUCTION

Falls and their resulting injuries are a major public health problem in the growing older population and present one of the most frequent complications among patients with stroke (1-3). The risk of falling is twice as high in patients with stroke than in people without stroke (4). Previous studies suggested that falls occur in approximately 23% to 50% of patients with stroke (5). The incidence of falling after an acute stroke is thought to be 14%–22% during hospitalization. Six months following the discharge from hospital, approximately half of stroke survivors have experienced a fall. More than a half of these falls resulted in injuries that required medical attention (6).

Falls and their resulting injuries during hospitalization cause serious problems and consequences for patient outcomes, quality of life and increased healthcare costs (7, 8). Falls may result in physical injuries including fractures, brain damage, bruising, bleeding and even death (9, 10). Approximately 5% of falls result in fractures and serious injuries (11). The psychological consequence of falling is related to fear of falling and subsequent activity restriction, reduced mobility, social isolation and depression (12, 13). Additionally, falls are associated with prolonged recovery period, increased length of hospitalization, greater healthcare expenses and legal consequences (14, 15).

To prevent these negative consequences for patients, preventive strategies are needed for patients at high risk of falls. Assessment of the risk of falling and the overview of the risk factors of falls, allow us good prevention of falls and of the serious consequences of falls as adverse events, and this leads to the increase of patient safety in the hospital settings. After examining the types of falls, spacious and timelines guidelines, work environment and organization of work in health departments can be improved. Good organization of nursing in health care facilities can significantly influence the success of prevention and treatment of patients, and improve the quality of work and satisfaction of both patients and employees.

Our objectives in the present study were fall risk assessment and risk factors identification, related to falls among stroke patients.

## METHODS

This was a retrospective cohort study that included 217 neurological patients with acute stroke who have experienced fall during hospitalization in the Special Hospital for Cerebrovascular Diseases "Sveti Sava" in Belgrade, Serbia. The study period was five years, from January 1, 2011 to December 31, 2015. This research was approved by the Hospital Ethics Committee and Ethics Committee of the Medical Faculty of the University of Novi Sad.

The data were collected from medical documentation. From the Book of adverse events, the following data were collected: the number of patients' falls, the sequence of falls in the hospital, place, time and circumstances of the fall and the consequences of the fall. From the history of the disease, patient data were collected related to: demographic characteristics (gender and age), medical diagnosis at the admission and at the discharge, the existence of other medical diagnosis, neurological examination results, evaluation of weakness (left or right), the therapeutic treatment of a patient, duration of hospitalization and the outcome of hospitalization (discharge of the patient, transfer to other department or institution, death and epicrisis of the death cause).

Morse Fall Scale was used to estimate a likelihood of falling for hospitalized patients. MFS is composed of six items. Each item was evaluated by score ranging from 0 to 30 points, and the total MFS score ranged from 0 to 125 points, with higher numbers representing an increased risk of fall. The six main items measured by the MFS are: "History of falling; immediate or within 3 months" [No=0, Yes=25]; "Secondary diagnosis" [No=0, Yes =15]; "Ambulatory aid" [Bed rest/nurse assist=0, Crutches/Cane/Walker=15, Grasps on furniture for support to walk=30]; "Intravenous therapy" [No=0, Yes=20]; "Gait/Transferring" [Normal/Bed rest/Immobile=0, Weak=10, Impaired=20]; and "Mental status" [Oriented to own ability=0, Forgets limitations=15] (16). According to the assessment of risk of falling, three categories of risk can be obtained: low risk (MFS < 25 points); moderate risk (MFS 25-44 points) and high risk (MFS 45 points and higher).

Data processing and analysis were performed using the software Statistical Package for Social Science (SPSS), version 21.0. All data of interest were presented and analyzed by statistical methods appropriate for the data type. The Chi-square test was used to compare proportions between groups. The student's t-test was used to compare-the mean values of continuous variables. The analysis of variance (ANOVA), or Kruskal-Wallis assay respectively, was used to analyze the score obtained by Morse questionnaire for each patient. Testing of the connection of attributive characteristics frequency was conducted by usage of Pearson's and Spearman's correlation coefficients. Statistical significance was defined at $p < 0.05$.

## RESULTS

During the study period of five years, a total of 15077 patients with a diagnosis of acute stroke were hospitalized, and the study included 217 (1.4%) patients who experienced a fall during hospitalization. There was no statistically significant trend in the incidence of falls during the reporting period ($\chi^2 = 0.35$; $p = 0.55$). The mean age of the patients was 74.2±8.9 years (range 48–93 years). Among them, 78.8% were men, 77.9% had ischemic stroke and 7.8% had brain hemorrhage. Respondents, according to the neurological findings, had weakness mostly on the left side of the body (64.5%), and, as a nursing diagnosis, had a deficit in self-care (62.2%). Respondents were mostly hospitalized in the intensive care units (48.8%), a fall usually occurred by the bedside (62.7%) and during the night (56.7%). The mean length of hospitalization was 19.6±7.6 days (range 8–49 days).

According to the Morse scale, 18% of the respondents had previous falls, and 6% of respondents experienced repeated falls in the hospital. 100% of the subjects had other medical diagnosis. Respondents mostly did not use mobility aids (63%), but if they did, the most common form of assistance was the help from other persons,(19.4% of respondents). Most respondents had intravenous therapy (92%) and they were not able to move (41.0%). More than a half (59%) of respondents were not oriented in time and space or forgot recommended limits (Table 1).

**Table 1.** Distribution of respondents according to Morse Fall Scale of risk factors

| Variables | | n | % |
|---|---|---|---|
| History of falling | no | 178 | 82.8 |
| | yes | 39 | 18.0 |
| Secondary diagnosis | no | 0 | 0 |
| | yes | 217 | 100 |
| Ambulatory aid | none/bed rest/nurse assist | 179 | 82.5 |
| | crutches/cane/walker | 25 | 11.5 |
| | furniture | 13 | 6.0 |
| Intravenous therapy | no | 17 | 7.8 |
| | yes | 200 | 92.2 |
| Gait | normal | 63 | 29.0 |
| | weak | 89 | 41.0 |
| | impaired | 65 | 30.0 |
| Mental status | oriented to own ability | 89 | 41.0 |
| | overestimates/forgets limitations | 128 | 59.0 |

The mean value for the risk of falling, obtained by the use of the MFS scale, was 56.9 points (SD=21.9), with a minimum of 15 and a maximum of 125 points. 3.7% of the patients had low risk, 18.4% had moderate risk and 77% had high risk of falls. The average value of the Morse score in male subjects was 55.8 ± 21.5, whereas among the female subjects it was 60.8 ± 23.3 (Table 2).

**Table 2.** Crude and adjusted odds ratios (OR) of the risk factors for death in neonates due to NRDS

| Morse Fall Scale risk | Total | | Men | | Women | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| low risk | 8 | 3.7 | 7 | 4.1 | 1 | 2.2 |

| Morse Fall Scale risk | Total | | Men | | Women | |
|---|---|---|---|---|---|---|
| | n | % | n | % | n | % |
| moderate risk | 40 | 18.4 | 37 | 21.6 | 3 | 6.5 |
| high risk | 169 | 77.9 | 127 | 74.3 | 42 | 91.3 |
| Mean score | 56.9±21.9 | | 55.8±21.5 | | 60.8±23.3 | |
| $\chi^2$=6.21 p=0.04 | | | | | | |

There was a statistically significant difference in the mean values of the Morse score between the studied groups with respect to gender, age, length of hospitalization and level of care. Women, older respondents and those who were hospitalized for period longer than 22 days and who had higher levels of care, had higher values of Morse score (Table 3).

**Table 3.** Morse score in relation to the characteristics of the respondents

| Variables | n | x̄ | sd | med | min | max | p |
|---|---|---|---|---|---|---|---|
| **sex** | | | | | | | |
| male | 171 | 55.8 | 21.5 | 50 | 15 | 125 | p<0.05 |
| female | 46 | 60.8 | 23.3 | 50 | 15 | 125 | |
| **age** | | | | | | | |
| up to 65 and 65 | 34 | 44.3 | 16.8 | 45 | 15 | 75 | p<0.001 |
| more than 65 | 183 | 59.2 | 22 | 55 | 15 | 125 | |
| **diagnosis** | | | | | | | |
| Endocranial infarctus | 169 | 57.4 | 20.5 | 50 | 15 | 125 | |
| hemorrhage | 17 | 56.5 | 18 | 55 | 35 | 105 | p=0.730 |
| other | 31 | 54 | 30.5 | 50 | 15 | 125 | |
| **neurological deficit** | | | | | | | |
| right-sided weakness | 43 | 50.6 | 18.6 | 50 | 15 | 110 | |
| left-sided weakness | 140 | 58.6 | 19 | 60 | 15 | 125 | p=0.11 |
| no lateralization | 34 | 57.6 | 33.5 | 50 | 15 | 125 | |
| **the level of care** | | | | | | | |
| general | 82 | 47 | 21.2 | 45 | 15 | 115 | |
| semi-intensive | 18 | 66.4 | 22 | 70 | 15 | 115 | |
| intensive | 106 | 62.9 | 20 | 60 | 35 | 125 | |
| special intensive | 6 | 63.3 | 23.6 | 57,5 | 45 | 110 | p<0.001 |
| special | 5 | 49 | 7.4 | 50 | 40 | 60 | |
| **length of hospitalization** | | | | | | | |
| up to 16 days | 69 | 47.1 | 20.9 | 45 | 15 | 115 | |
| 16 – 22 days | 85 | 60.3 | 20.3 | 60 | 15 | 125 | p<0.001 |
| longer than 22 days | 63 | 62.9 | 21.8 | 60 | 35 | 125 | |

T test was used for comparison of a correlation of medication use and Morse score. The results showed a statistically significant effect of the therapy applied in the form of infusion solutions, sedatives and anti-diabetics (p<0.05) (Table 4). The median of time until the fall of patients with periodic monitoring was 3 days (95% CI: 2.2 – 3.8), and of

patients with increased surveillance was 5 days (95% CI: 3.9 – 6.1), while in patients with the constant supervision was 6 days (95% CI: 3.8 - 8.2) (Figure 16). There was a statistically significant difference in the time before the observed outcome, in relation to the level of supervision ($\chi^2$ =16.051; p<0.001).

**Table 4.** Correlation of medication and the risk of falling

| Therapy | Medication | $\bar{x}$ | sd | med | min | max | p |
|---|---|---|---|---|---|---|---|
| antiedematous thrombolysis | yes | 55.0 | 22,5 | 50.0 | 15.0 | 125.0 | 0.139 |
| | no | 59.4 | 20.9 | 60.0 | 15.0 | 125.0 | |
| infusion | yes | 59.9 | 20.9 | 55.0 | 15.0 | 125.0 | 0.001 |
| | no | 31.6 | 18.2 | 27.5 | 15.0 | 65.0 | |
| antihypertensives, cardiotonics, bronchodilators | yes | 56.3 | 21.0 | 50.0 | 15.0 | 125.0 | 0.587 |
| | no | 58.0 | 23.9 | 60.0 | 15.0 | 125.0 | |
| diuretics | yes | 54.3 | 23.0 | 52.0 | 15.0 | 115.0 | 0.335 |
| | no | 57.7 | 21.6 | 50.0 | 15.0 | 125.0 | |
| sedatives / anticonvulsant | yes | 59.9 | 22.2 | 60.0 | 15.0 | 125.0 | 0.027 |
| | no | 53.3 | 21.1 | 50.0 | 15.0 | 115.0 | |
| anticoagulants | yes | 56.1 | 21.1 | 50.0 | 15.0 | 115.0 | 0.703 |
| | no | 57.3 | 22.5 | 50.0 | 15.0 | 125.0 | |
| antiaggregational therapy | yes | 55.3 | 20.1 | 50.0 | 15.0 | 115.0 | 0.572 |
| | no | 57.3 | 22.4 | 50.0 | 15.0 | 125.0 | |
| antibiotics | yes | 60.7 | 21.1 | 60.0 | 15.0 | 125.0 | 0.082 |
| | no | 55.1 | 22.1 | 50.0 | 15.0 | 125.0 | |
| antidiabetics / insulin | yes | 51.3 | 16.9 | 55.0 | 15.0 | 125.0 | 0.015 |
| | no | 58.6 | 23.0 | 55.0 | 15.0 | 125.0 | |
| corticosteroids | yes | 52.2 | 30.2 | 40.0 | 15.0 | 125.0 | 0.495 |
| | no | 57.3 | 21.1 | 50.0 | 15.0 | 125.0 | |

## DISSCUSION

Our research shows that falls of neurological patients who had stroke are represented at a frequency of 1.4%, which is a different result in comparation with numerous studies that showed larger average representation of falls. Studies (17, 18) showed that 39% of stroke patients fell at least once during hospitalization, and 24% of patients experienced repeated fall. Aizen et al. (19) showed that 15.6% of patients with stroke experienced a fall, while research conducted in Australia (20) showed that the incidence of fall was 6.3%. Since the data on the incidence of falls for this study were collected from the Book of adverse events, it is possible that not all the falls were recorded, as it was confirmed by the data collection conducted at the Semi-intensive care unit, where the Book of adverse events is used for records keeping back from the 2015. Another possible reason is a well-designed plan of care according to the existing nurse diagnoses in relation to the "risk of falling" and well implemented preventive measures.

In relation to sex, significantly more respondents were male. Most studies show that women tend to fall more frequently (19, 20), although some studies show results identical to ours (21, 22). Research showed that female respondents had higher Morse score, which is similar to the results of our research.

These researches showed that the major predictor of falls is age, and that there was a statistically significant difference in the mean Morse score values among respondents who were younger than 65 and those older than 65 years. Older respondents had higher values of Morse score and experienced falls more frequently.

The results of our study show that the use of disability aids contributes most to the value of Morse score and contributes statistically to the risk of falling. The study confirms the hypothesis that the leading causes of neurological patients to fall are internal, and that the risk factors are associated with motor and cognitive impairments related to the underlying disease. In their study, Sehested and Severin (23) stated that

a third of respondents who experienced a fall, used some kind of disability aids in order to move or to transfer from a chair or from a wheelchair. Bugdayci et al. (20) showed that a half of the respondents experienced a fall during a walk, and the other half during the transfer. Deandrea et al. study (24), which included 24 studies in the meta-analysis, came to a result that a leading risk factor was a history of falls, followed by the use of disability aids and by cognitive disorders. Risk factor for falls was also the age of the respondent, where, for every increase in the age of 5 years, a risk of falling increased by 10%. Ma et al.(25) identified in their study 28 risk factors for falls, including altered mental status, special needs in self-care and, to a lesser extent, the age of patients and usage of some drugs (sedatives, hypnotics). Oliver et al. (26) identified five of the most common risk factors for falls in hospitalized patients: unstable walking, frequent need to go to toilet, confusion, use of a sedative - hypnotic agents and the history of falls. Hee-Yoo et al. in Korea (27) showed that neurological patients have higher risk of falling, especially those patients who cannot move. The results of study conducted by Hee-Sung et al. (22) support the fact that patients who are partially dependent on assistance of others fall more frequently compared to patients who are independent.

Most neurological patients' falls occur during the acute phase of the disease (20, 22, 28). Our study shows that the median value of falls was on the fourth day, while most of respondents have experienced a fall in the second day of hospitalization (16.6% of respondents). This interesting fact points to the need to improve care plan in terms of prevention of falls in the acute phase of the disease. Other studies indicate that the extent of the fall was in the range from the first day to 26th day of hospitalization. Some studies show the highest number of falls in the second and third week of hospitalization (19).

In relation to the fall location, our study showed that the most of falls occurred beside sickbed (62.7%), while the sickroom, the hall and the restroom were on the second place as a significant fall locations with a low frequency (12%), which, to some extent, corresponds to the third hypothesis that the falls occur most frequently in hospital room, bedside the sickbed. Similar results were shown by other authors: from 41% to 44% falls occur beside the sickbed (19, 20), while 80% occur in the patient's room (29). Some studies showed that most falls of neurological patients occur in the bathroom, 68% (30).

The study results show that, in relation to the period of day, 56.7% of respondents experienced a fall during the night. Studies showed that the fall usually happens during the night (26, 32) in about 60% of cases. Some studies give different results. Multicentre studies proved that the falls also occur during the day in a period between 6 and 20 , which is explained by the increased need to get up from the bed to perform self-care activities (22, 20, 28). The presented results provide a significant contribution to the improvement of the organization of work in the medical department and in the working environment.

The research of Huey (31), conducted in the United States in several hospitals, showed that falls depend on organization of hospitals, on the number and training of nurses at the medical departments, on communication between the staff and patients, on a good pain control, on health-educational work with patients and on a good training in a transfer of patients and informing patients about the medication they take. It also stated that the reduction in the rate of falls is affected by hospital cleanliness, by tranquility and peace during night hours and by the possibility of timely nurse call (31, 32). These research results are valuable for improving measures to prevent falls. The literature data, according to Ackly and Ladwig (32), confirm that the administration of drugs may be a risk factor for falls (antihypertensives, diuretics, acetinholinesteraze inhibitors, tricyclic antidepressants, opioids).

**Strengths and limitations**

Retrospective analysis of medical documentation and small sample sizes have limited the generalisability of these study findings. Although numerous studies have identified risk factors for falls among hospitalized patients, there are few studies that specifically investigate the risk factors for falls among hospitalized patients with stroke. The results of this study can be the basis for the implementation of the new scales for assessing the risk of falls, as well as for new measures of prevention of the falls in neurological patients in the hospital settings, which will increase the quality of health care and treatment of these patients and help us to achieve the prestigious goal of maximum patient safety.

## CONCLUSION

The most common risk factors for falls according to Morse scale are: the presence of other medical diagnosis, the use of disability aids while walking, the use of intravenous therapy, disorientation in time and space, and the largest contribution to Morse score comes from using disability aids while walking and transferring patients. Greater risk of falling is observed in older neurological patients with ischemic type of stroke and weakness on the left side of the body, patients with longer hospitalization and those with a higher level of care. The degree of neurological deficit was negatively correlated with the fall.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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# THE ROLE OF HMG COA REDUCTASE INHIBITORS ON THE PROGRESSION OF CORONARY ARTERY DISEASE: FOCUS ON PREDICTION MODEL

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# ULOGA HMG COA INHIBITORA REDUKTAŽE NA PROGRES KORONARNE ARTERIJSKE BOLESTI: FOKUS NA MODEL PREDVIĐANJA

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## ABSTRACT

*Currently, an integrated site-specific and patient-specific comprehensive predictive model of plaque progression in various CVD is not available. In this study, we considered medical records of 256 patients obtained within the EU H2020 SMARTool project which is carefully designed to collect the features from various domains relevant for disease which are used in everyday clinical practice. The database contains detailed information of patients with suspected CAD disease regarding the clinical status, risk factors, routine blood analyses, CAD morphology and progression and current therapy. Results showed that there was statistically significant difference of values of this parameter for the SMARTool patients with and without disease progression, measured at the follow-up, $F(1,250)=33.39$, $p < 0.001$, while the CAD Score in the follow-up is significantly different from the score measured at the initial time point, $F(1,254)=76.244$, $p < 0.001$. The significant interaction of statins is achieved with aspirin $F(1,252)= 3.921$, $p=0.049$, while interactions with other medicaments are insignificant for CAD Score. The results showed that there was no significant interaction of statins and dyslipidemia, $F(1,251)=0.877$, $p = 0.350$. Also, there was no significant interaction of statins and hypertension, $F(1,245)=0.283$, $p=0.596$. The CAD score in the baseline was significantly different among patients who were further prescribed with therapy than those who were not, and this trend remained unchanged after a given time period, i.e. those patients who were at risk had progression in addition to statins, but the combination of statins and aspirin was shown as effective in decreasing the CAD Score. The Random Forest classifier applied on 24 selected features is the most reliable among all tested ML algorithms for the prediction of CAD progress.*

***Keywords**: prediction, statins, atherosclerosis, plaque, coronary artery disease.*

## SAŽETAK

*Trenutno, integrisani, specifični i za pacijenta sveobuhvatni prediktivni model progresije plaka kod različititih kardiovaskularnih bolesti (KVB) nije dostupan. U ovoj studiji razmatrali smo medicinske podatke 256 pacijenata dobijenih u okviru projekta EU H2020 SMARTool koji je pažljivo dizajniran da prikupi osobine iz različitih domena relevantnih za bolesti koje se koriste u svakodnevnoj kliničkoj praksi. Baza podataka sadrži detaljne informacije za pacijente sa sumnjom na KVB posmatrajuci klinički stanje, faktore rizika, rutinske analize krvi, morfologiju i progresiju KVB i trenutnu terapiju. Rezultati su pokazali da postoji statistički značajna razlika između vrednosti ovog parametra za SMARTool pacijente sa i bez progresije bolesti tokom pracenja, $F(1,250)=33.39$, $p<0.001$, dok se CAD ocena u sledećem značajno razlikuje od rezultata merenja u početnoj vremenskoj tački, $F(1,254)=76.244$, $p <0.001$. Značajna interakcija statina se primećuje sa aspirinom $F(1,252)=3.921$, $p=0.049$, dok su interakcije sa drugim lekovima neznatne. Rezultati su pokazali da ne postoji značajna interakcija statina i dislipidemije, $F(1,251)= 0.877$, $p=0.350$. Takođe, ne postoji značajna interakcija statina i hipertenzije, $F(1,245)=0.283$, $p=0.596$. CAD status u osnovnoj liniji bio je značajno različit kod pacijenata koji su bili dalje na propisanoj terapiji od onih koji nisu, a ovaj trend je ostao nepromenjen i nakon određenog vremenskog perioda, tj. oni pacijenti koji su bili u riziku imali su progresiju i pored upotrebe statina, ali kombinacija statina i aspirina pokazala se kao efikasna u smanjenju progresije CAD-a. Random Forest primenjen na 24 odabrane karakteristike pacijenata je najpouzdaniji među svim testiranim ML algoritmima za predviđanje progresije CAD.*

***Ključne reči:** predviđanje, statini, ateroskleroza, plak, koronarna arterijska bolest.*

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**ABBREVIATIONS**

**ATS**-Atherosclerosis; **CAD**-Coronary artery disease;
**CVD**-Cardiovascular diseases; **CHD**-Coronary heart disease;
**ML**-Machine Learning; **KDD**-Knowledge Discovery in Databases;
**NB**-Naive Bayes; **BN**-Bayes Nets; **DT**-Decision Tree; **RF**-Random forest;
**SVM**-Support Vector Machine; **ANN**-Artificial Neural Network;sdf

## INTRODUCTION

Atherosclerosis (ATS) is a local process (the high risk plaque) in the context of a systemic disease (the high risk patient). Medical therapy and control of lifestyle, diet related and clinical (dyslipidemia, hypertension, diabetes) risk factors are firmly established as fundamental aspects of CAD patient-specific management. On the other hand, emerging data and technologies have raised again the interest in locally targeted diagnostic and therapeutic approaches. Multiple factors contribute to plaque formation/progression/complication, by a complex interaction between biological and mechanical elements (1, 2).

Both artery-specific and patient-specific factors are known to contribute and even if some have been proposed as predictors of ATS formation and progression, none of them has fulfilled so far all the criteria stated by the American Heart Association (AHA) for comprehensive evaluation of novel markers of CVD risk (2). Coronary imaging has been demonstrated, by large scale trials and extensive meta-analysis studies, to improve diagnostic accuracy of obstructive CAD, assessment of therapy efficacy, prediction of CAD progression rate and prediction of plaque-related MACE (major adverse cardiovascular event) (5). In CAD stratification, non invasive CCTA imaging has been shown to outperform nonimaging conventional risk factors, clinical models and scores as well as current molecular biomarkers, including lipoproteins (4-6).

Currently, an integrated site-specific and patient-specific comprehensive predictive model of plaque progression is not available, and, even though several algorithms have been developed for primary and secondary prevention of CAD and CAD associated CHD (coronary heart disease), there is no platform that integrates all local and systemic risk factors into a unique tool for clinical decision support in stratification, diagnosis, prediction and treatment through a personalized approach.

## MATERIAL

### Patients and study protocol

In this study, we considered medical records of 256 patients obtained within the EU H2020 SMARTool project (15). The database contains detailed information of patients with suspected CAD disease, clinically evaluated at the time of the first CT coronary angiography (CTCA) scan in the *EVINCI* project (16) and then clinically re-evaluated by a second CTCA scan in the *SMARTool* project.

For all patients, the relevant factors that affect the clinicians' decisions on therapy modulation were recorded in two-time moments, baseline and follow-up. The average inter-scan period between these time moments was 6.23±1.35 years. These features ranged from clinical data, CAD risk factors, biohumoral data, therapy as well as from CAD Morphology data:

#### i. Clinical Data

The available clinical variables include patient's age, gender, current symptoms.

#### ii. CAD risk factors

As the main risk factors, the following parameters were analyzed: family history of CHD, presence of diabetes mellitus, arterial hypertension, dyslipidemia, obesity in terms of BMI class, and being a current or former smoker.

#### iii. Blood Test Data

The laboratory data available include standard blood test parameters such as fasting glucose, creatinine, uric acid, total cholesterol, HDL and LDL levels, triglycerides, alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), gamma-glutamyl transferase (GammaGT), but also specific inflammatory markers such as hs-C reactive protein, interleukin 6, leptin, ICAM1, VCAM1 and hs-cardiac Troponin T.

### iv. CAD Morphology

The CAD morphology is described with several features: number of plaques, maximal stenosis class (0 = no stenosis, 1 = <30%, 2 = 30-50%, 3 = 50-70%, 4 = >70%), number of stents, indication about CABG, and CAD Score. CAD Score (3) is a composite score of patient-specific CAD severity and its calculation is based on the number of plaques and its location, percent of stenosis and composition.

### v. Therapy

It is indicated for every patient in the database which medicaments were prescribed from the following list: ARB, ACE Inhibitors, Diuretics, ß-Blockers, Calcium Antagonists, Nitrates, Oral Antidiabetics, Insulin, Aspirin, DAPT, Traditional Anticoagulants, Novel Anticoagulants and Statins. Data about accurate name of medicaments and dosage were available only for statins.

### vi. CAD Progression

CAD progression (YES/NO) is visually classified according to changes in % maximal diameter stenosis between baseline and follow up from baseline severity class to a higher severity class at follow up scan in at least one of the plaque-related constricted coronary segments of each patient.

## Development of predictive models for prediction of CAD progress

In order to prescribe the optimal medical treatment, it is of great importance for clinicians to have the ability to predict in a reliable manner how the use of medicaments could affect the progress of the disease. For the CAD, it is of particular interest to predict how using of statins and its dosage influence on the disease progress.

Machine Learning (ML), as a branch of Artificial Intelligence, represents the set of algorithms that have the ability to learn from data, i.e. to detect hidden patterns in databases with the aim to use generated knowledge to predict new outputs of the system (7). It is very useful approach which can be applied in various domains of problems where collections of relevant real data could not be explored in a deterministic way.

The problem of CAD progress prediction was modeled as a classification problem by using the real *SMARTool* database which is carefully designed to collect the features from various domains relevant for thedisease which are used in everyday clinical practice. It is consisted of 256 patients described with 34 features described in Section 2.1.

There is a standardized process to perform classification - Knowledge Discovery in Databases (KDD) (8). Two important steps which very often need to be resolved within the ML model development are feature selection and resolving of the imbalanced classification.

**Imbalanced classification problems.** Imbalanced distribution of patients for various classification problems in health domains of data is a very often phenomenon. Generally, events with lower frequency could negatively affect the ML models and its misclassifying could lead to unfavorable decisions and results in heavy costs. SMOTE algorithm (10) is shown as a good choice when data set consist of a small number of minority instances.

**Feature selection.** Identification of significant feature subsets is important for two main reasons. First, the complexity of solving the classification problem is reduced and data quality is improved by ignoring the irrelevant features. In that way, the risk that minority class samples become discarded in the case of imbalance classification problems could be significantly reduced (11). In this study, we used the following feature selection methods: ReliefF [14], MRMR [7] and wrapper technique for feature selection (14) with genetic algorithm (GA) as a search method.

**Machine learning algorithms**. The learning task is the core task in the process of prediction model development. A set of representative supervised machine learning algorithms is used in this study: Naive Bayes (NB), Bayes Nets (BN), Decision Tree (DT), Random forest (RF), ADABoost with DT as a weak classifier (AdaBoost), Support Vector Machine (SVM) and Artificial Neural Network (ANN) (7).

**Validation of prediction models.** In order to validate the performance of the prediction models and evaluate their generalization ability the 10 times repeated 10-fold cross-validation technique was applied in this study**.** In each 10-fold cross-validation run, we partitioned the available data into 10 folds. Then, iteratively, each of these folds was used as a test set while the remaining folds were used to train a prediction model, which was evaluated on the test subset. The average classifier performance on all test sets provides a real measure of the classifier's performances on the particular discrimination problem.

Accuracy (1), sensitivity (2), specificity (3), precision (4), kappa statistic (5) and the area under the ROC curve (AUC) (6) are standard metrics used to measure the classifiers' performances. These values are calculated on the basis of the entries of the confusion matrix that contains information about the actual and predicted classification (Table 1).

**Table 1.** Confusion matrix

| | | Predicted | |
|---|---|---|---|
| | | Positive | Negative |
| Actual | Positive | TP | FN |
| | Negative | FP | TN |

$$ACC = \frac{(TP + TN)}{(TP + FN + FP + TN)} \quad (1)$$

$$SENS = \frac{TP}{(TP + FN)} \quad (2)$$

$$SPEC = \frac{TN}{(TN + FP)} \quad (3)$$

$$PREC = \frac{TP}{(TP + FN)} \quad (4)$$

$$kappa = \frac{p_o - p_e}{1 - p_e} \quad (5)$$

### Statistical analysis

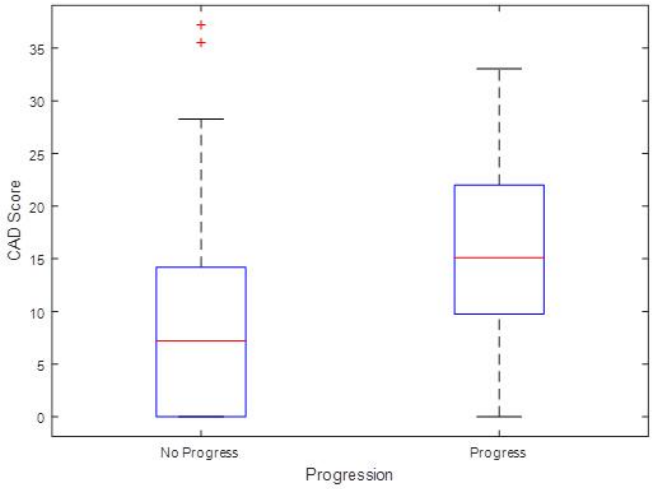
The aim of statistical analyses was to examine whether using of statins has the influence on the change of CAD score over two-time moments (baseline and follow-up) based on the *SMARTool* database. Several experiments were designed in order to reveal significant interaction of statins with other factors: The influence of statins with or without other medicaments on the change of CAD Score and the influence of statins on the change of CAD Score in the case of different risk factors. The statistical analyses were performed by applying the Mixed ANOVA tests within the SPSS software.

## RESULTS

### Statistical assessment of the role of statins on the CAD progress

With the aim to perform all abovementioned tests and gain the relevant results about the role of statins on the CAD progress, we firstly proved that CAD Score is an important parameter for monitoring the CAD and its progression. Namely, based on ANOVA test, there is statistically significant difference of values of this parameter for the *SMARTool* patients with and without disease progression, measured at the follow-up, $F(1,250) = 33.39$, $p < 0.001$ (Figure 1). As a measure of CAD severity correlated with the progress of the disease progression, the CAD Score was used to test how its values were changed after using the targeted therapy.

**Figure 1.** Significance of CAD Score values difference between progress and no progress groups, measured in follow-up



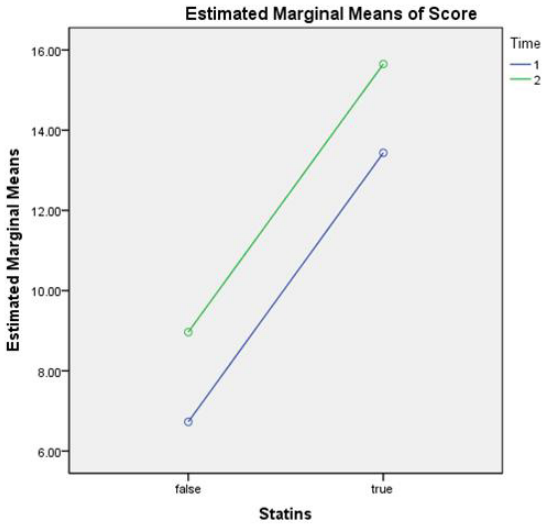

Values are mean ± standard error of the mean (SEM), n=5 per group,
*denotes a significant difference p<0.05, **denotes a significant difference p<0.01.

### The influence of statins on the change of CAD Score

Mixed ANOVA for the sample of 256 patients was applied. CAD Score in the follow-up was significantly different from the score measured in the initial time point, $F(1,254) = 76.244$, $p < 0.001$.

There is also a significant main effect of statins, which in our case means that patients that used statins had significantly higher CAD score in comparison to patients that did not use this medicament, $F(1, 255) = 33.829$, $p<0.001$. However, there was no significant interaction of statins over time, $F(1,254)=0.002$, $p=0.964$ (Figure 2).

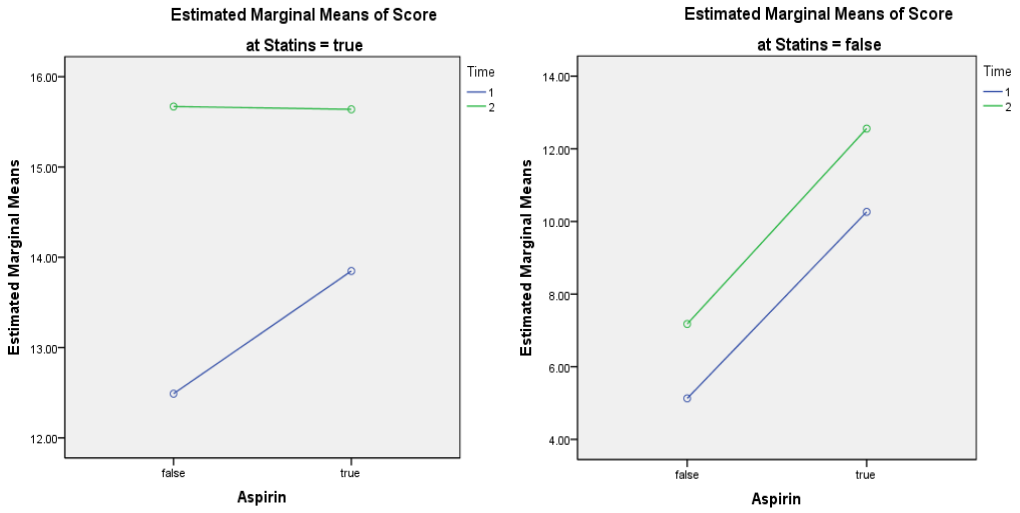
**Figure 2.** Interaction of statins as between factor and time as within factor for the estimation of CAD Score change




### CAD score changing based on combination of statins with other group of medicaments

The significant interaction of statins was achieved with aspirin $F(1, 252) = 3.921$, $p = 0.049$, while interactions with other medicaments were insignificant for CAD Score. We can report that there was a significant difference between conditions where the statins were used are those where they were not used (Figure 3).

**Figure 3.** Interaction of statins and aspirin as between factors and time as within factor for the estimation of CAD Score change




This figure demonstrates that if patients used statins and aspirin simultaneously, the change of CAD Score was significantly lower than in the case when only statins were used.

**CAD Score changing over two time points based on statins and risk factors (such as dyslipidemia and hypertension[1]) as two independent factors**

The results showed that there was no significant interaction of statins and dyslipidemia, $F(1,251) = 0.877$, $p = 0.350$. Also, there was no significant interaction of statins and hypertension, $F(1,245) = 0.283$, $p = 0.596$.

**Prediction of the CAD progress and the role of statins**

Different combinations of data domains with statins and its dosage were examined in order to develop the most accurate data mining model for the prediction of CAD progress. Models were developed and tested based on follow-up data.

The problem of the prediction of disease progress required resolving the issue of unbalanced classification, since 153 patients had the progress, and 103 did not. To overcome of this problem, the SMOTE algorithm was used.

**Table 2**. The performances of classifiers

| | **Algorithms** | **ACC** | **Sens** | **Spec** | **Prec** | **kappa** | **AUC** |
|---|---|---|---|---|---|---|---|
| Case 0 | NB – ReliefF(20) | 0.625 | 0.616 | 0.643 | 0.779 | 0.2335 | 0.713 |
| | **BN – all attributes** | **0.789** | **0.930** | **0.5** | **0.792** | **0.4735** | **0.763** |
| | DT – MRMR(9) | 0.748 | 0.837 | 0.568 | 0.8 | 0.415 | 0.712 |
| | **RF – MRMR(24)** | **0.787** | **0.891** | **0.574** | **0.811** | **0.49** | **0.795** |
| | **AdaBoost** | **0.766** | **0.831** | **0.607** | **0.813** | **0.4439** | **0.742** |
| | SVM – GA(20) | 0.781 | 0.913 | 0.512 | 0.793 | 0.4612 | 0.715 |
| | ANN – MRMR(27) | 0.723 | 0.74 | 0.69 | 0.83 | 0.405 | 0.77 |

[1] Data are extremely unbalanced for other risk factors which make them unsuitable for the statistical analysis.

**Table 2.** The performances of classifiers

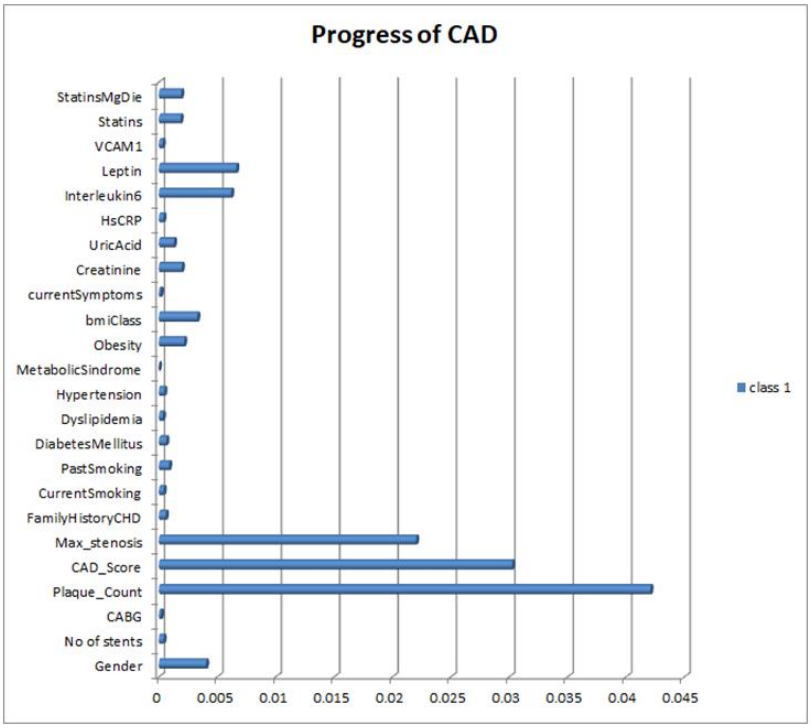
| | Selected attributes |
|---|---|
| ReliefF (20) | CAD Score, Max stenosis class, No of plaques, BMI class, LDL, Obesity, Total Cholesterol, Gender, Past smoking, ICAM1, Uric Acid, Dyslipidemia, **Statins**, Family history CAD, HDL, **Statins MgDie**, Age, Current symptoms, Leptin, Hypertension |
| MRMR (24) | VCAM1, CABG, Hs – CRP, Metabolic Syndrome, Current Smoking, Creatinine, Max stenosis class, Dyslipidemia, Obesity, Hypertension, Gender, Diabetes Mellitus, No of plaques, family history CAD, Past smoking, No of stents, **Statins**, Current symptoms, Interleukin6, BMI class, CAD Score, Uric Acid, **Statins MgDie**, Leptin |
| GA (20) | Gender, Age, No of stents, No of plaques, Max stenosis class, Family history CAD, Current smoking, Dyslipidemia, Obesity, BMI class, Current symptoms, ALT, GammaGT, Total Cholesterol, LDL, HDL, Interleukin6, Cardiac Troponin-T, **Statins, Statin MgDie** |

Every ML algorithm was combined with all of feature selection algorithms with the aim to test it in a more comprehensive way. Only the best results of the classifiers' performances for this task are presented in Table 2 and the selected attributes are listed in Table 3.

From Table 2 it can be observed that almost all algorithms achieved good results for the sensitivity parameter which demonstrates the ability of classifiers to reliably distinguish patients that had the progress of disease. Based on the AUC parameter, which is an effective and combined measure of sensitivity and specificity and which provides the general ability of model to correctly perform classification, RF-MRMR(24) model achieved the best results (Accuracy = 78.7%, Sens = 0.891, Spec = 0.574, Prec = 0.811, kappa = 0.49, AUC = 0.795). This means that RF classifier based on 24 features selected by MRMR algorithm is the selected model for the prediction of CAD progress. These features are listed in Table 3 and it is important to note that statin as well as its dosage are among selected attributes by every algorithm for feature selection.

The averaged contributions of attributes for the prediction of CAD progress by this model are presented in Figure 4.

**Figure 4.** The averaged contributions of attributes for the model RF – MRMR(24)



## CONCLUSION

Atherosclerosis is multifactorial, so the application of statin for disease correction is only one therapeutic step. The CAD score in the baseline was significantly different among patients who were further prescribed with therapy than those who did not, and this trend remained unchanged after a given time period, i.e. those patients who were at risk had progression in addition to statins. If there was an interaction of the application of the statin over time, this would have a positive impact on the CAD score, in that the difference between the CAD scores between the two groups of subjects would decrease. However, the combination of statins and aspirin was shown as effective in decreasing the CAD Score.

More specifically, we have demonstrated based on the *SMARTool* dataset that using of statins in combination with other factors related to CAD could be used to predict whether the CAD will progress or not. Wide series of data obtained from patients medical records were assembled in order to provide the comprehensive data set able to capture the possible disease manifestations. The results showed that the Random Forest classifier applied on 24 selected features was the most reliable among all tested ML algorithms to predic whether the patient will have CAD progress or not.

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# BITTER LEAF (VERNONIA AMYGDALINA) MODULATES NITROBENZENE-INDUCED RENAL DAMAGE IN RATS VIA SUPPRESSION OF OXIDO-INFLAMMATORY ACTIVITIES

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# GORKI LIST (VERNONIA AMIGDALINA) MODULIRA NITROBENZENOM INDUKOVANO OŠTEĆENJE BUBREGA KOD PACOVA PREKO SUPRESIJE OKSIDO-INFLAMATORNIH AKTIVNOSTI

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## ABSTRACT

*Renal diseases have been documented as one of the massive health challenges, ranked as the 12th most common cause of death globally. This study was carried out to assess the chemo-preventive effects of Vernonia amydalina on nitrobenzene mediated renal damage in rats. Rats were exposed to 100 mg/kg body weight of nitrobenzene via oral administration and treated with 200 mg/kg body weight (BW) and 400 mg/kg BW of methanol leaf extract of Vernonia amydalina (MLVA) and Vitamin E for 14 consecutive days. Nitrobenzene significantly induced a renal injury with a significant increase in the serum levels of urea and creatinine with the concomitant altered serum electrolyte profile. Also, nitrobenzene mediated the oxidative stress and lipid peroxidation with a significant increase in the renal level of malondialdehyde (MDA), hydrogen peroxide ($H_2O_2$), with a concomitant decrease in the level of reduced glutathione (GSH), Catalase (CAT) and Superoxide dismutase (SOD). Furthermore, an inflammation was observed in the nitrobenzene-treated rats with the elevated level of nitric oxide (NO) and myeloperoxidase (MPO). However, the treatment with methanol leaf extract of Vernonia amydalina reversed all the nitrobenzene-associated renal damage, electrolyte imbalance, oxidative stress, lipid peroxidation, inflammation and altered antioxidant defence system. Taken together, methanol leaf extract of Vernonia amydalina offers protection which may be beneficial for the treatment and management of kidney diseases or other related disorders via enhancing the serum electrolyte homeostasis, protecting the structural integrity of the kidney, antioxidant, anti-inflammatory mechanisms.*

**Keywords**: *Renal damage, Nitrobenzene, oxidative stress, Vernonia amydalina, Inflammation, Electrolyte imbalance*

## SAŽETAK

*Za bubrežne bolesti je dokazano da su jedan od najvećih zdravstvenih izazova i zauzimaju 12. mesto među najčešćim uzrocima smrtnosti na globalnom nivou. Ova studija je izvedena kako bi procenila hemo-preventivne efekte Vernonia amigdaline kod nitrobenzenom indukovanog ostećenja bubrega kod pacova. Pacovi su bili izloženi 100mg/kg telesne težine, nitrobenzenu preko oralne primene i tretirani sa 200mg/kg telesne težine i 400 mg/kg telesne težine sa metanolnim ekstraktom lista Vernonie amigdaline (MLVA) i vitaminom E, 14 uzastopnih dana. Nitrobenzen je značajno izazvao oštećenje bubrega sa znatnim povećanjem serumskih nivoa uree i kreatinina sa istovremenim izmenjenim serumskim elektrolitnim profilom. Takođe, nitrobenzen posreduje u oksidativnom stresu i lipidnoj peroksidaciji sa značajnim povećanjem nivoa malondialdehida (MDA) u bubrezima, vodonik peroksida ($H_2O_2$), sa istovremenim smanjenjem u nivou smanjenog glutationa (GSH), katalaze (CAT) i superoksid dismutaze (SOD). Osim toga, zapaljenje je bilo primećeno kod pacova tretiranih nitrobenzenom sa povišenim nivoom azotnog oksida (NO) i mijeloperoksidaze (MPO). Međutim, tretman sa metanolnim ekstraktom lista Vernonie amigdaline promenio je celokupno oštećenje bubrega povezano sa nitrobenzenom, neravnotežu elektrolita, oksidativni stres, lipidnu peroksidaciju, inflamaciju i izmenjeni antioksidativni odbrambeni system. Sve u svemu, metanolni ekstrakt lista Vernonie amigdaline pruža zaštitu koja može biti korisna za lečenje i tretiranje bolesti bubrega ili drugih povezanih poremećaja preko pospešivanja serumske elektrolitne homeostaze, štiteći pri tome strukturni integritet bubrega, antioksidativne i anti-inflmatorne mehanizme.*

**Ključne reči:** *oštećenje bubrega, nitrobenzene, oksidativni stres, Vernonia amigdalina, inflamacija, neravnoteža elektrolita*

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## INTRODUCTION

Renal damage has been described as one of the massive health issues, the stream of which continues to rise unavoidably. In 2015, the report of Global Burden of Disease Study, the kidney disease was recorded as the 12th most common cause of death which accounts for 1.1 million deaths throughout the World (1). The total kidney damage mortality rate was also documented to have increased by 31.7% over the last 10 years making it one of the fastest increasing foremost causes of death, along with other diseases like diabetes and dementia. Renal damage has been recorded to be a major problem considered by morphological damage of intracellular organelles, cellular necrosis, mitochondrial vacuolization, followed by functional modifications which include a reduction in the synthesis of protein, reduced glutathione (GSH) depletion, lipid peroxidation (LPO) and mitochondrial damage (2).

Nitrobenzene (NB) has been regarded as one lethal air contaminant with a proven ability to cause cancer in animals. According to the 1986 Cancer guidelines, it was classified as a group B2 chemical, as likely a human carcinogen (3). It is water-insoluble pale-yellow oil with an almond odor like substances used in industry for making varieties of chemicals such as aniline, analgesics, dye, polish for shoes and other useful products. During the metabolism of NB, intermediates such as nitrosobenzene (NOB) and phenylhydroxylamine (PH) are formed and play an important role in the progression of Nitrobenzene carcinogenesis (4). Certain high concentration of Nitrobenzene was also reported to be found in the liver, brain, blood and stomach following accidental nitrobenzene poisoning in humans (4).

Phytoextracts or their products have been reported to improve the kidney damage induced by free radicals, thereby protecting it against any forms of functional modifications and injury. This protection against the renal damage, and other reported pharmacological abilities like antioxidant, neuroprotective and anti-inflammatory activities validate the exhaustive scientific investigation into their nephroprotective properties (5). The herb known as bitter leaf (*Vernonia amygdalina*) is a shrub or small tree which is known to reach about 23 feet in height when fully grown. Bitter leaf is known for having a grey or brown coloured bark, which has a rough texture even when peeled. The herb is an indigenous African plant; which grows in most parts of the African continent (6). Bitter leaf (*Vernonia amygdalina*) has been reported to have hypoglycemic, anti-diabetic and anticholesterol properties (7, 8). Besides, the extract ameliorates haematotoxicity in rats exposed to petroleum hydrocarbon feeds (9).

Based on the aforementioned information, this study was carried out to examine the adverse effects of nitrobenzene in the kidney of *Wistar albino* rats via the evaluation of serum electrolytes, renal function tests, renal redox status, and post-mortem histopathology of the renal tissue. Furthermore, we aimed to assess the ameliorative potentials of methanol leaf extract of *Vernonia amygdalina* against renal toxicities associated with nitrobenzene exposure.

## MATERIALS AND METHODS

### Chemicals/ Reagents

High purity (> 99.7 %) Nitrobenzene was obtained from BDH chemical Poole England. Vitamin E (Alpha Tocopherol) is a product of Embassy pharmaceuticals, Nigeria. Urea, creatinine assay kits are diagnostic kits from Randox. All other chemicals are of the analytical grade and were obtained from Analar BDH Limited, Poole, England and Sigma-Aldrich Co. St Louis, Missouri, USA.

### Collection of plant material and preparation of extract

Fresh leaves of *Vernonia amygdalina* were collected at the staff quarters in Kings University, Odeomu, Osun State. The leaf has been identified at IFE-Herbarium of Botany Department, Obafemi Awolowo University, Ile-Ife with Voucher number, IFE-16885. The *Vernonia amygdalina* leaves were washed and air-dried at room temperature in the Biochemistry laboratory, Kings University, Nigeria and pulverized using an electric blender. The powdered leaf was defatted in n-hexane using Soxhlet apparatus. Thereafter, methanol extract was prepared by soaking the defatted leaf in 90 % methanol for 72 hours. The resulting mixture was then filtered and the filtrate was concentrated on water bath. The concentrated extract was lyophilized using Bosch freeze drying machine. The full chemical identification and bioactive compounds of *Vernonia amygdalina* have been earlier reported by Oladunmoye et al (10) using gas chromatography-mass spectrometry (GC-MS). The technique reveals methyl-2-O-benzyl-d-arabinofuranoside, phytol, hexadecanoic acid, ethyl ester, squalene and 9, 12, 15, octadecatrienoic acid as more abundant compounds (>85 % abundance) while N-[2-(dimethylamino)-5-pyrimidinyl] benzene sulfonamide, 9, 12, 15 and octadecatrien-1-ol, p-Menth-4(8)-en-9-ol have lesser abundance (<12 % abundance) compounds.

### Experimental animals

Thirty mature, 4-5 months old male Wistar strain albino rats with body weight range 140 – 160 g were used in the study. The rats were sourced and raised at the Biochemistry breeding colony of the Biochemistry Unit, Department of Chemical Sciences, Kings University, Ode-Omu, Osun State, Nigeria. The animals were kept under the ambient standard conditions (25 ± 2 °C and relative humidity of 50 ± 15 %) in stainless steel cages and metabolic wastes were cleaned twice daily. The rats were allowed to acclimatize to these conditions for fourteen days and were exposed to 12 hrs daylight and darkness cycle, fed with commercially available rat pellet and water *ad libitum.* The experiment was carried out in accordance with the current rules and guidelines that have been established for the care of the laboratory animals (11). The rats were randomised into five groups containing six rats each.

Group A: received distilled water daily and served as the Control.
Group B: received 100 mg/kg Nitrobenzene orally.
Group C: received 100 mg/kg Nitrobenzene and 200 mg/kg *Vernonia amygdalina*
Group D: received 100 mg/kg Nitrobenzene and 400 mg/kg *Vernonia amygdalina*
Group E: Received 100 mg/kg Nitrobenzene and 400 mg/kg Vitamin E

The treatments were administered to the rats through oral gavage for 14 consecutive days.

### Preparation of liver homogenates

The kidneys were immediately excised and blotted to remove blood stains. They were cleansed and rinsed in 1.15 % KCl on ice to remove haemoglobin, then weighed. They were then chopped into bits and homogenized in four volumes of the homogenizing buffer (10 mM potassium phosphate buffer, pH 7.4) using a Teflon homogenizer. The homogenates were centrifuged at 12,500 g for 15 minutes in a cold centrifuge (4 °C) to obtain the post mitochondrial fractions which were collected and used for biochemical analyses.

### Measurement of biochemical markers

The protein content of the homogenates was determined using Bovine Serum Albumin (BSA) as a standard in the protocol described by Lowry et al. (12). Urea, and creatinine acid were determined colometrically following the instruction of the assay kits manufacturer. The Nitric oxide (NO) level was assessed by the procedure reported by Green et al. (13). Myeloperoxidase (MPO) activity in the homogenate was quantified following the method of Granell et al. (14). Superoxide dismutase (SOD) activity was evaluated following the inhibition of adrenaline auto-oxidation in a basic milieu as described by Misra and Fridovich (15). Lipid peroxidation was evaluated by monitoring the level of malondialdehyde (MDA) using the procedure reported by Varshney and Kale (16). The reduced glutathione (GSH) content in the brain samples was determined using the protocol reported by Buetler et al. (17). Catalase (CAT) activity was determined following the protocol documented by Clairborne (18) using hydrogen peroxide ($H_2O_2$) as a substrate. Hydrogen peroxide generation was assayed by oxidation of ferrous ions and sorbitol colour amplification system using the method of Wolff (19).

### Histological examination

The kidney sections were fixed in 10 % formalin and embedded in paraffin wax. Thin sections (7–9 mm thickness) of the kidney tissues were cut and dewaxed in xylene, hydrated in decreasing percentage of alcohol and stained with hematoxylin and eosin (H & E). They were differentiated in 90% alcohol and cleared in xylene. These stained sections were observed under the microscope for histopathological analysis.

### Statistical analysis

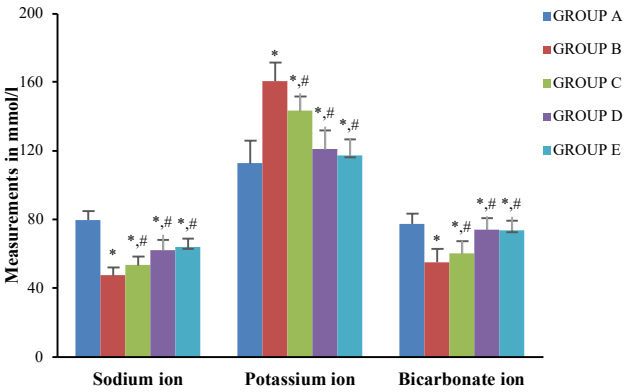
The results obtained were expressed as the mean ± standard deviation (mean ± SD) and analysed using one-way analysis of variance (ANOVA) with the aid of SPSS 22.0 computer software package (SPSS Inc; Chicago, U.S.A) to compare the experimental groups followed by Bonferroni's post-hoc test. Values of $P<0.05$ were considered significant.

## RESULTS

### Effects of MLVA on the serum electrolyte profile of rats treated with nitrobenzene

Serum electrolytes profile levels in the experimental animals are shown in figure 1. Nitrobenzene exposure caused a significant increase in potassium ion and a significantly reduced sodium ion and bicarbonates as compared to the control. However, the treatment with 200 and 400 mg/kg body weight of MLVA or vitamin E caused a drastic reversal in the serum level of the electrolytes close to normal.

**Figure 1.** Effect of MLVA on the serum electrolyte profile of rats treated with nitrobenzene

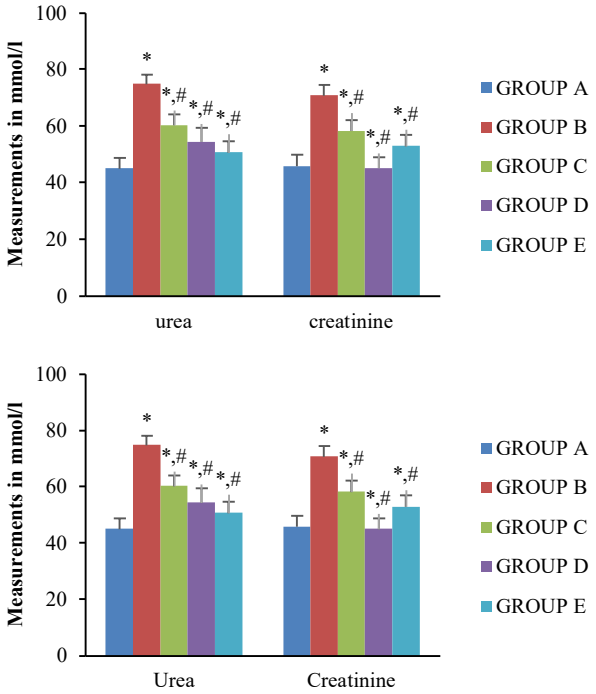



Data are given as the mean ± SD of rats per group. n=6. MLVA: methanol leaf extract of *Vernonia amygdalina*, *: Values differ significantly from the group A (control) ($P < 0.05$). #: Values differ significantly from the group B.

### Effects of MLVA on the serum renal metabolites of rats treated with nitrobenzene

Figure 2 shows that the rats exposed to 100 mg/kg body weight of nitrobenzene (group B) showed a significant increase in the serum concentration levels of urea and creatinine as compared to the control (group A). The serum elevated values of urea and creatinine were reversed significantly toward normal in a dose dependent manner in the rats treated with 200 and 400 mg/kg body weight of MLVA or vitamin E.

**Figure 2.** Effect of MLVA on the serum renal metabolites (urea and creatinine) in rats treated with nitrobenzene

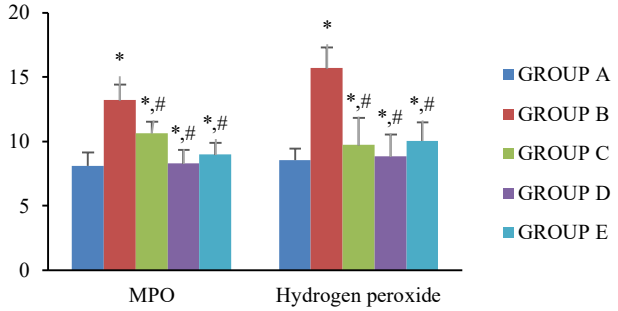



Data are given as the mean ± SD of rats per group. n=6. MLVA: methanol leaf extract of *Vernonia amygdalina*, *: Values differ significantly from the group A (control) ($P < 0.05$). #: Values differ significantly from the group B.

## MLVA mitigated oxidative stress in the kidney of rats treated with nitrobenzene

Figure 3 showed the results of renal oxidative stress biomarker ($H_2O_2$ generation) carried out in the experimental rats. There was a marked increase in the level of $H_2O_2$ generation in the kidney of rats that received nitrobenzene only when compared with the control group. Upon the treatment with 200 and 400 mg/kg of MLVA or Vitamin E, there was a significant decrease in $H_2O_2$ generation level confirming the free radical and ROS scavenging ability of the extract.

**Figure 3.** Effect of MLVA on the renal myeloperoxidase (MPO) and hydrogen peroxide generation ($H_2O_2$) levels in rats treated with nitrobenzene.

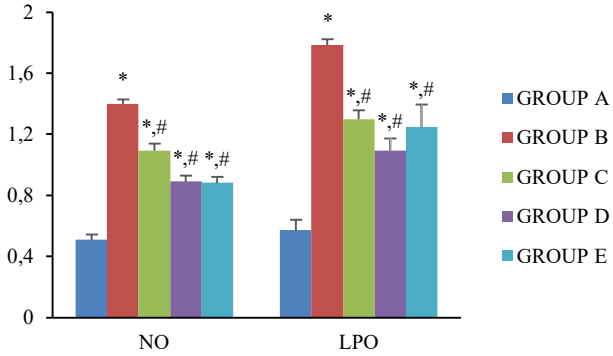



MPO activity (Units/mg protein); $H_2O_2$ (µmole/mg protein). Data are given as the mean ± SD of rats per group. n=6. MLVA: methanol leaf extract of *Vernonia amygdalina*, *: Values differ significantly from the group A (control) ($P < 0.05$). #: Values differ significantly from the group B.

## Effects of MLVA on the renal inflammatory markers of rats treated with nitrobenzene

The effects of MLVA on inflammation in the kidney of the experimental rats were evaluated by measuring MPO activities and NO concentration level. The rats that received nitrobenzene only, demonstrated a marked increase in NO level and MPO activities when compared with the control (Figure 3 and 4). However, the treatment with 200 and 400 mg/kg of MLVA or Vitamin E significantly attenuated both NO and MPO levels in the kidney when compared with the control.

**Figure 4.** Effect of MLVA on the renal nitric oxide (NO) and Lipid peroxidation (LPO) level in rats treated with nitrobenzene




Lipid peroxidation (µmol MDA/mg protein); NO level (Unit/mg protein). Data are given as the mean ± SD of rats per group. n=6. MLVA: methanol leaf extract of *Vernonia amygdalina*, *: Values differ significantly from the group A (control) ($P < 0.05$). #: Values differ significantly from the group B.

## MLVA inhibited lipid peroxidation in the kidney of rats treated with nitrobenzene

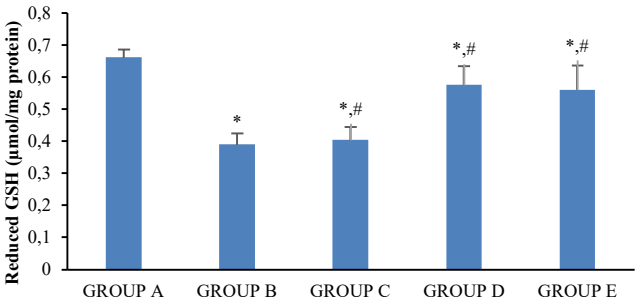
The result of renal lipid peroxidation assayed in this experiment was depicted in Fig. 4. There was a marked increase in MDA level (the index of lipid peroxidation) in the rats that received nitrobenzene only when compared with the control group. However, the treatment with 200 and 400 mg/kg of MLVA or Vitamin E inhibited the peroxidation with a significant decrease in MDA level.

## Effects of MLVA on the reduced glutathione level and antioxidant enzymes activities in the kidney of rats treated with nitrobenzene

Figure 5-7 shows the glutathione (GSH) level and antioxidant enzymatic activities of CAT and SOD in the kidney of the experimental rats. The application of nitrobenzene alone caused a significant decrease in the GSH level and a decline

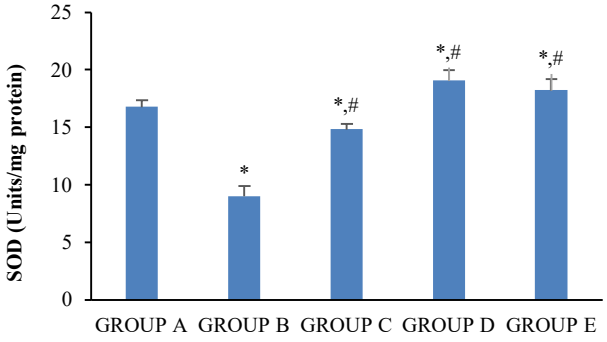
in CAT and SOD activity when compared with the control. However, the treatment with 200 and 400 mg/kg of MLVA or Vitamin E significantly increased the GSH level and enhanced the activity of all the enzymes.

**Figure 5.** Effect of MLVA on the renal reduced glutathione level in rats treated with nitrobenzene



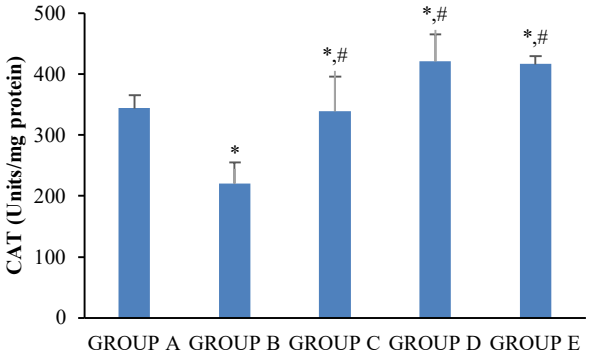

Data are given as the mean ± SD of rats per group. n=6. MLVA: methanol leaf extract of *Vernonia amygdalina*, *: Values differ significantly from the group A (control) ($P < 0.05$). #: Values differ significantly from the group B.

**Figure 6.** Effect of MLVA on the renal superoxide dismutase (SOD) activities in rats treated with nitrobenzene.



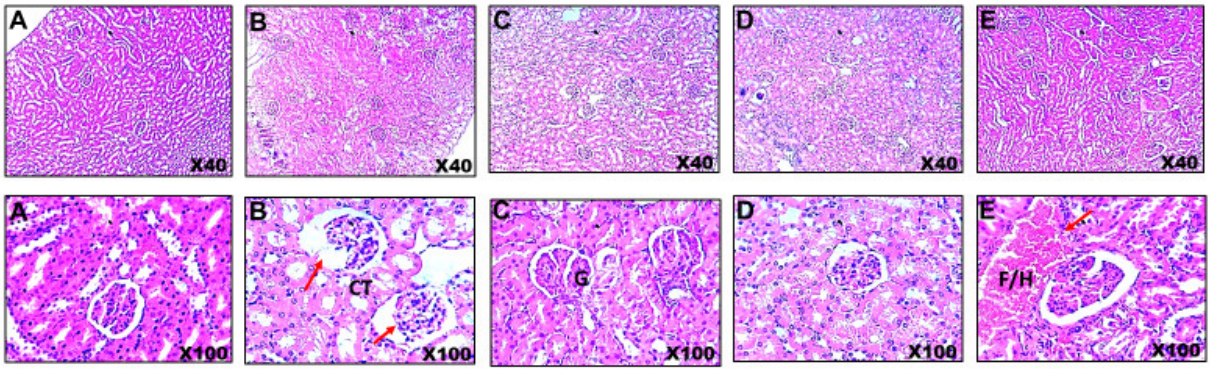

Data are given as the mean ± SD of rats per group. n=6. MLVA: methanol leaf extract of *Vernonia amygdalina*, *: Values differ significantly from the group A (control) ($P < 0.05$). #: Values differ significantly from the group B.

**Figure 7.** Effect of MLVA on the renal catalase (CAT) activities in rats treated with nitrobenzene




Data are given as the mean ± SD of rats per group. n=6. MLVA: methanol leaf extract of *Vernonia amygdalina*, *: Values differ significantly from the group A (control) ($P < 0.05$). #: Values differ significantly from the group B.

**Figure 8.** Photomicrographs of the renal cortex showing the panoramic views of Kidney general micromorphological presentations in Adult Wistar rats across the study groups A-E. Hematoxylin and Eosin stain (X40 and X100 Magnification). The Renal Corpuscles, Renal glomeruli, Macula densa, Distal and Proximal convoluted tubules and the Bowman's capsule are observable across the study groups.



**Protective effects of MLVA on the histological alterations in the kidney sections of nitrobenzene-induced renal damage in rats**

The histological alterations seen with the light microscope in the kidney sections of the experimental rats are shown in Figure 8. The collagen of the parietal layer of Bowman's capsule and the basal membrane of a distal tubule are observable from the photomicrographs. The groups B and E showed mild fibrosis and haemorrhage characterized by presence of red inflammatory cells (red thick arrow) but no other varying degrees of the renal injury except for marked shrinkage of the glomerulus observable in the group B, the groups A and D showed no marked degenerative changes however, mild focal sclerosis of the glomerulus, mild widening of the Bowman's space and hyper cellularity are all observable across the groups B, C and E. There is no observable hyaline arteriosclerosis, no observable interstitial fibrosis, no interstitial inflammation observed across the groups A and D. The areas of pathomorphological observations are indicated by red arrows.

## DISSCUSION

The role played by the kidneys in maintaining the balance of fluid and electrolytes in the serum becomes clear when considering the consequences on electrolytes in the rats with the acute renal failure. The levels of electrolytes such as potassium, sodium, magnesium, calcium and bicarbonates can all be affected and lead to an array of signs and symptoms. When there is a malfunction of the kidneys, the balance of fluid and electrolytes levels can be altered, leading to the imbalance of certain electrolytes resulting in serious implications or consequences (20).

In this study, the serum level of sodium and bicarbonate ions was significantly decreased due to the exposure of nitrobenzene to the experimental rats. The concentration of these electrolytes is essential for maintaining the fluid and acid-base balance and for proper renal function (21). Healthy kidneys alter the excretion of sodium in the urine to prevent certain diseases like hypernatremia. However, the administration of various concentrations of methanol leaf extract of *Vernonia amygdalina* or Vitamin E significantly increased the serum levels of both sodium and bicarbonate ions levels. This result suggests that the extract can enhance the electrolyte homeostasis and may be beneficial in the renal physiological function.

Furthermore, nitrobenzene mediated a marked increase in potassium ion concentration in the serum. Potassium ion is the major cation of the intracellular fluid, while about 10 % of the total body potassium is also found in the extracellular one. Any changes in the serum potassium ions level are known to have serious health implications like hyperkalemia which is associated with symptoms such as abdominal cramping, fatigue, muscle weakness, paralysis and cardiac arrest (22,23). The treatment with various concentrations of methanol leaf extract of *Vernonia amygdalina* or Vitamin E significantly decreased the potassium ion level in the serum. This suggests the protective effect of the extract on peroxidation of the polyunsaturated fatty acids in the membrane which delocalized Na-K ATPase from basolateral to apical membrane resulting in the electrolyte imbalance (24).

The kidney injury is characterized by the elevation of the kidney metabolites (urea and creatinine) in the serum. The observed increase in the serum levels of urea and creatinine after the exposure to nitrobenzene in this study may be a result of leakage of the kidney membrane, excessive protein intake or impairment in the kidney functions (25,26). The urea and creatinine levels were further determined to establish the source of the perturbation. However, the improved renal function following the treatment with the leaf extract suggests the protective effect of *Vernonia amygdalina* on the kidney. The ameliorative effect of the leaf extracts on the kidney function could be attributed to constituent phytochemicals such as terpenoids (27), saponins (28), flavonoids (29) and tannins with known renoprotective properties. Flavonoids could confer renoprotection against glomerulonephritis, diabetic nephropathy, and chemically induced kidney insufficiency (29).

In this study, the exposure of rats to nitrobenzene caused a marked increase in the level of renal $H_2O_2$. Biotransformation of nitrobenzene has been reported to generate free radicals and reactive oxygen species which in turn, alters the antioxidant system and finally results in the oxidative stress and macromolecule damage (30). $H_2O_2$ can be rapidly decomposed into oxygen and water and this may produce hydroxyl radicals ($^{\bullet}OH$) that can initiate lipid peroxidation and cause DNA damage (31). However, the treatment with graded doses of methanol leaf extract of *Vernonia amygdalina* or Vitamin E significantly mitigated the hydrogen peroxide generation in the kidney. This can be attributed to the antioxidant and free radical scavenging effects of the extract. Plants have been proven to be an important source of natural antioxidants and phytochemicals that mitigated the oxidative stress instigated by an increase in free radicals/ROS and help in the treatment of many human diseases.

Similarly, the administration of nitrobenzene to the rats caused a significant increase in lipid peroxidation as indicated by a marked level of MDA in the kidney. Lipid peroxidation has been reported to play a critical role in carcinogenesis (32). The process produces some byproducts which are highly toxic to the cells. These toxic byproducts include MDA and 4-hydroxynonenal. They can easily attack cellular targets such as proteins and DNA leading to genetic mutations and ultimately to carcinogenicity (33). However, the groups treated with graded doses of methanol leaf extract of *Vernonia amygdalina* or Vitamin E showed a significant reduction in the level of malondialdehyde when compared to the animals treated with nitrobenzene only. The observed decrease in lipid peroxidation in the rats treated with methanol leaf extract of *Vernonia amygdalina* could be due to its ability to scavenge hydroxyl and peroxyl radicals.

Nitrobenzene induced an inflammation in the kidney of the experimental rats as evidenced by a marked increase in the level of NO and MPO activity. This observation indicates the involvement of aggravated inflammatory response in nitrobenzene-induced hepatotoxicity. NO is a toxic defense molecule synthesized by inducible nitric oxide synthase (iNOS) in many cell types involved in immunity and inflammation. The treatment with various doses of methanol leaf extract of *Vernonia amygdalina* or Vitamin E significantly inhibited the inflammatory process in the renal cells. This agrees with the previous report that the extract has anti-inflammatory properties (34).

One of the primary functions of antioxidant and free radical scavenging enzymes such as CAT, and SOD is to protect biological cells against the free radical attacks and oxidative stress. This study revealed that the administration of nitrobenzene caused a significant reduction in the activity of these enzymes in the rats. The observed reduction in these enzymes activities may be a result of overwhelming detoxification activities of the enzymes by conjugating with the free radicals/ROS and other toxic by-products to enhance their excretion. However, there was a marked increase in CAT, and SOD in the rats treated with graded doses of methanol leaf extract of *Vernonia amygdalina* or Vitamin E. Many scientific reports have proven that one of the protective mechanisms of actions of plant extracts is via upregulation of these endogenous antioxidant enzymes (35,36).

Furthermore, a depletion in the GSH level was observed in the nitrobenzene treated rats. This significant decrease in the GSH level might have been due to GSH usage by the detoxifying enzyme (GST) and it may be responsible for an increase in lipid peroxidation (37). The free radical mediated tissue damage can be inhibited or alleviated by ensuring the redox balance to decrease the oxidative stress. On the other hand, the rats treated with *Vernonia amygdalina* or Vitamin E display a marked increase in the GSH level. This agrees with the previous report of Oladele *et al* (38) who documented that *Vernonia amygdalina* caused a reversal to the decreased GSH level induced by nitrobenzene. This suggests that the protective effect of *Vernonia amygdalina* extract involves maintenance of the antioxidant capacity in preventing the renal cells against the oxidative damage.

## CONCLUSION

The result from this study revealed that nitrobenzene induced the renal damage with an evidence of the altered serum electrolyte homeostasis and a significant increase in the serum level of renal metabolites (urea and creatinine). A marked increase in hydrogen peroxide with a concomitant decrease in the GSH level, CAT and SOD activities also confirmed the oxidative stress in the kidney. Furthermore, an inflammation was observed in the kidney with the increased level of NO and MPO with the concomitant increased level of MDA confirming lipid peroxidation. However, the treatment with various concentrations of methanol leaf extract of *Vernonia amygdalina* reversed all the nitrobenzene-associated renal injury, oxidative stress, lipid peroxidation, inflammation and altered antioxidant defence system. Similarly, histological observations showed that the extract was capable of not only preventing but actually reversing pathomorphological changes of the nitrobenzene-induced kidney injury such as mild fibrosis and hemorrhage characterized by presence of red inflammatory cells. Taken together, methanol leaf extract of *Vernonia amydalina* exhibited the renal protection which may be beneficial for the treatment and management of kidney diseases or other related disorders via enhancing the serum electrolyte homeostasis, protecting the structural integrity of the kidney, antioxidant, anti-inflammatory mechanisms.

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# COMPARATIVE EVALUATION OF BGR-34 AND SITAGLIPTIN IN DIABETIC SUBJECTS - OPEN LABELLED RANDOMISED PARALLEL CLINICAL STUDY

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# KOMPARATIVNA EVALUACIJA BGR-34 I SITAGLIPTINA KOD DIJABETIČARA - OTVORENA RANDOMIZOVANA PARALELNA KLINIČKA STUDIJA

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**Running Title:** BGR-34 & Randomised Parallel Clinical Study

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## ABSTRACT

*This article is mainly concerned with the simultaneous open model clinical study of the drug named sitagliptin, a potent hyperglycaemic drug against a novel entity of the natural origin BGR-34 in diabetic subjects. This was a 3-month randomized, parallel, comparative study. One hundred subjects were planned to be included in the study. The patients were randomly divided into two groups and according to the appropriate sample size analysis, both groups consisted of 100 patients, following the inclusion and exclusion criteria. A total of 90 patients (both male and female) of the mean patient age 30-65 years with the type 2 diabetes were enrolled in the phase 4 of this study and then the data were analyzed on the basis of the different test which included HbA1c (glycated haemoglobin), RBS (random blood sugar), FBS (fasting blood sugar) and PPG (postprandial glucose) values. After completion of the data calibration, the results were analyzed and as a result 10-20% decreased values of HBA1C values accompanied with the RBS, FBS and PPG values were seen in the patients undergoing a 12-week course with BGR-34. Based on the results obtained in the present study, it can be concluded that BGR-34 is effective in reducing high blood sugar levels and this reflects that the BGR-34 therapy is more effective drug in the treatment of diabetes suggesting that it is better in efficacy, and reliability with little or no adverse effects.*

***Keywords*:** *diabetes mellitus, blood glucose regulator-34, DPP-4 inhibitors, hyperglycemia, sitagliptin.*

## SAŽETAK

*Ovaj rad se uglavnom bavi simultanom kliničkom studijom otvorenog tipa za lek Sitagliptin, koji je jedan jak hiperglikemijski lek nasuprot novog entiteta prirodnog porekla BGR-34 kod dijabetičara. Ovo je tromesečna randomizovana paralelna komparativna studija. Planirano je da 100 pacijenata bude uključeno u studiju. Pacijenti su nasumično podeljeni u dve grupe i prema odgovarajućoj analizi veličine uzorka, obe grupe su se sastojale od 100 pacijenata i pratile su kriterijume inkluzije i ekskluzije. Ukupno 90 pacijenata (muškaraca i žena), prosečno godište 30 – 65 godina sa dijabetesom tip 2 su bili uključeni u četvrtu fazu ove studije i onda su podaci analizirani na osnovu različitog testa koji je obuhvatio vrednosti HbA1c (glikolizirani haemoglobin), RBS (nasumični nivo šećera u krvi), FBS (nivo šećera u krvi pre jela) i PPG (postprandijalni nivo glukoze). Posle kompletiranja, kalibrisanih podataka, rezultati su analizirani i kao rezultat 10-20% smanjene vrednosti HBA1C praćene sa RBS, FBS i PPG vrednostima su primećene kod pacijenata koji su bili podvrgnuti BGR-34 u toku 12 nedelja. Zasnovane na rezultatima dobijenim u sadašnjoj studiji, može se zaključiti da je BGR-34 efikasan u smanjenju visokih nivoa šećera u krvi i ovo pokazuje da je terapija sa BGR-34 efikasniji lek u lečenju dijabetesa što sugeriše da je bolji što se tiče delovanja i pouzdanosti sa malo ili bez neželjenih dejstava.*

***Ključne reči*:** *dijabetes melitus, regulator-34 glukoze u krvi, DPP-4 inhibitori, hiperglikemija, sitagliptin.*

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## INTRODUCTION

Type 2 Diabetes Mellitus (T2DM) is not a new entity, which requires any introduction, as it has been affecting individuals globally for decades. The presentation of the aforementioned metabolic condition involves hyperglycemia and glucose intolerance which result from the lack of insulin secretion, defective insulin action or perhaps both (1). Such complications arise due to the derangements in the regulatory systems for storage and mobilization of the metabolic fuel, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from the defective insulin secretion, insulin action, or both. In addition to this, the intake of high calories and physical inactivity potentially increase the likelihood of T2DM (2). It has been found that around 366 million people had T2DM in 2011 (3) and it is expected to reach the number of 552 million by the year 2030. The incidence of T2DM results most commonly due to the environmental as well as lifestyle factors and varies geographically (4). Individuals aged between 45 years to 64 years are at a higher risk to be affected with this metabolic condition (5). T2DM can further trigger chances of developing macro- and micro-vascular complications associated with the elevated blood sugar such as cardiovascular diseases, peripheral vascular diseases, stroke, neuropathy, renal failure, retinopathy etc (6). Furthermore, various genetic, environmental and behavioural factors are responsible for the increased prevalence of diabetes mellitus (5). Urbanization, aging, physical inactivity, obesity, etc are exponentially resulting in the increased prevalence of diabetes mellitus worldwide. Numerous oral hypoglycaemic agents (OHA's) have been available at the market since quite a long time for the effective management of T2DM. One of the therapeutic modalities named "Sitagliptin", which belongs to the class of Dipeptidyl Peptidase-4 (DPP-4) inhibitor, has been found to be relatively effective in the management of T2DM with a low risk of hypoglycaemia. It brings about inhibition of DPP-4 enzyme. DPP-4 is a complex molecule that is expressed in various cell types. It selectively degrades the levels of two incretins i.e. Glucose-dependent insulinotropic polypeptide (GIP) and glucose-like peptide-1 (GLP-1) in the body that is released into the circulation after meals and potentiates the secretion of glucose dependent insulin from pancreatic β cells. GLP-1 also inhibits the secretion of glucagon and gastric emptying. The activation of incretin receptor stimulates the secretion of insulin in the presence of elevated blood glucose. Sitagliptin lowers the activity of DPP-4 and increases the levels of intact GIP and GLP-1 following meals. Moreover, it reduces the blood glucose levels without causing significant hypoglycaemia (7). However, recently, there has been a trend of opting for traditional medicines instead of allopathic medication for the management of various chronic diseases and T2DM is not an exception. There are several works in the literature that are reviewed by many authors of herbal drugs for the treatment of diabetes [8]. Recently, The Council of Scientific & Industrial Research (CSIR) has developed a herbal drug "BGR-34", a Blood Glucose Regulator which comprises 34 vital plants. It maintains blood glucose, relieves symptoms, decreases the occurrence of diabetic complications and is highly safe and reliable.

The current study assessed the safety and efficacy of BGR-34 (620mg) in patients with the type 2 diabetes mellitus against Sitagliptin (100mg) with the inadequate glycaemic control on diet and exercise.

## SUBJECTS AND METHODS

### Study designs & Intervention

The current study was a randomized, parallelgroup, comparative study conducted over a period of 12 weeks in order to determine the efficacy and safety of the new molecular entity, BGR-34 at a tertiary care teaching hospital of North India. The Indian population was selected for the study in which the subjects were divided into two groups – Group 1 and Group 2 where the patients were receiving 100 mg of Sitagliptin once a day and 620 mg of BGR-34 once a day respectively for the duration of 12 weeks. In addition to this, the patients received counseling on a diet consistent with the American Diabetes Association recommendations for the study entry. There were a total of three followup visits along with the screening and baseline visit. The study subjects were assessed for any kind of side effects and safety during each and every visit. The screening data were reviewed to determine the subject eligibility. It was the responsibility of the principal investigator to educate or inform the subjects about the study and its outcomes. The study protocol was approved under the Gian Sagar Medical College and Hospital's Ethics Committee and was in compliance with the Declaration of Helsinki. A written informed consent was obtained from the patients who met all inclusion criteria and none of the exclusion criteria. After the statistical analysis and interpretation of the data, all the records were registered and reported appropriately to the associated higher authorities.

### Study endpoints

The primary endpoint was to assess the efficacy of BGR 34 by achieving the diabetic control. This was assessed by a reduction in the levels of glycosylated haemoglobin (HbA1c) in the study subjects; the key secondary endpoints included the assessment of Fasting Blood Sugar (FBS), Random Blood Sugar (RBS) and Post-Prandial Blood Sugar (PPBS). The safety assessment data for adverse drug reactions (ADRs), physical examinations, vital signs and body weight were maintained throughout the study. The causality assessment of ADRs was based on the World Health Organization (WHO) – Uppsala Monitoring Centre (UMC) Causality assessment scale. The baseline laboratory tests were performed before initiation of the study period such as alanine aminotransferase, aspartate aminotransferase, total bilirubin, alkaline phosphatase, creatinine, haematology and urinalysis.

### Study population

For the enrolment of the subjects in this double blind study, screening was performed. The enrolled subjects should be – the Indian population aged 30-65 years; diagnosed with T2DM; Fasting Blood Sugar (FBS) ranges from 126 mg/dl – 200 mg/dl; Post-Prandial Blood Sugar (PPBS) ranges from >200 mg/dl – 350 mg/dl; HbA1c ranges from 6.5 - >8.5 and not receiving any corticosteroids; Body Mass Index (BMI) > 25Kg/$M^2$ , the absence of any chronic disease; ready to give audio visual consent for the study participation. The individuals receiving insulin for the management of T2DM; suffering from the acute/chronic/ debilitating comorbid conditions; pregnant & lactating females; participation in any other clinical trial within the last 30 days; subjects with an allergy to investigation product; the individual meeting the inclusion criteria but unwilling to participate, were excluded from the study. The patients were randomly divided into two groups (Group 1 & Group 2) and according to the appropriate sample size analysis, both groups consisted of 100 patients, following the Inclusion and Exclusion criteria.

### Procedure

A total of 100 outpatients were enrolled from Gian Sagar Medical College and Hospital, Banur. The primary clinical measurements were the glycosylated hemoglobin (HbA1c) levels, Random Blood Sugar (RBS) levels, Fasting Blood Sugar (FBS) levels and Postprandial Glucose (PPG) levels tested within 3 months at the time of the enrollment and after each visit i.e. after the 4th week, 8th week and 12th week. Physicians collected the patients' demographic data and clinical characteristics at the baseline for all potentially eligible individuals. The study parameters were assessed using laboratory results.

### Statistical analyses

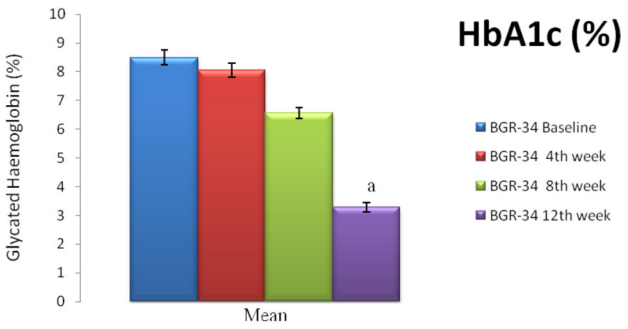
Continuous values were analyzed and represented as the mean, standard deviation (SD) and standard error mean (SEM). The demographic data and background information were recorded following the multivariate logistic regression analysis. SPSS (version 14.0) statistical software was used only for analyses. In this, the analysis was performed using the paired t tests, with a significance level of $p < 0.05$.

## RESULTS

### Glycosylated haemoglobin (HbA1c)

As depicted in Figure 1(A), the subjects receiving BGR-34 for the management of T2DM had a significant glycemic control over the period of 12 weeks. BGR -34 was able to reduce the mean HbA1c values significantly from the mean baseline HbA1c values (8.499% ± 0.25%) over the period of 4 weeks (8.061% ± 0.24%), 8 weeks (6.56% ± 0.196%) and 12 weeks (6.27% ± 0.111%).

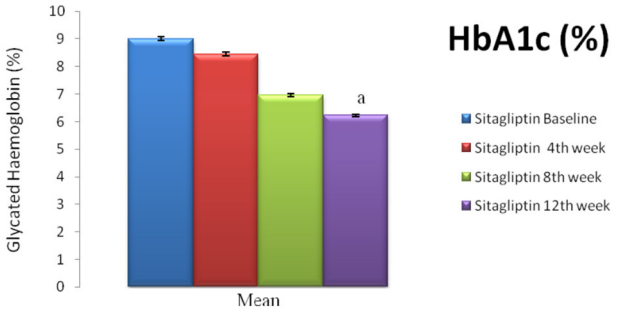
**Figure 1 (A).** Effect of BGR-34 on HbA1c over the period of 4, 8 and 12 weeks




a ($p<0.01$) vs. HbA1c % at the 12th week BGR-34

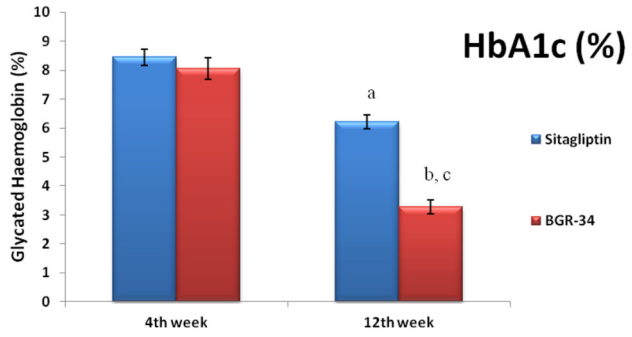
Similarily, sitagliptin also produced a similar effect by reducing the mean HbA1c values when compared to the mean baseline values of HbA1c (9.001% ± 0.073%) to 8.44% ± 0.074% (Over 4 weeks), 6.97% ± 0.057% (Over 8 weeks) and 6.22% ± 0.038% (Over 12 weeks) as represented in Figure 1 (B). A reduction in the levels of HbA1c in the BGR-34 group compared to their respective baseline value was significant with a p value of (<0.001). As represented in Figure 1(C), in the statistical comparison of glycemic control with BGR-34 and Sitagliptin over a period of 12 weeks, it was observed that the glycemic control with BGR-34 was more significant than Sitagliptin (P<0.001).

**Figure 1(B).** Effect of Sitagliptin on HbA1c over the period of 4, 8 and 12 weeks




a ($p<0.01$) vs. HbA1c % at the 12th week Sitagliptin

**Figure 1(C).** Effect of BGR-34 and Sitagliptin after 4 and 12 weeks on HbA1c (HbA1c) values in type 2 diabetic patients.

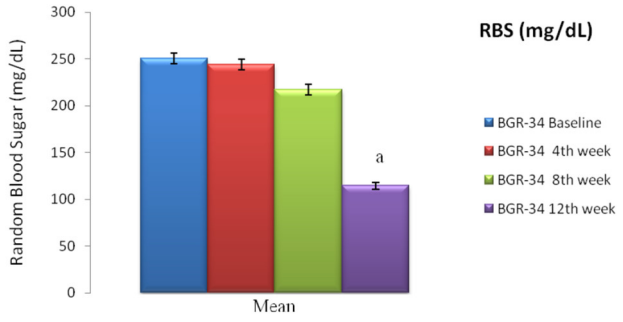



a ($p<0.05$) Vs HbA1c at the 4th week Vs 12th week (Sitagliptin)
b ($p<0.05$) Vs HbA1c at the 12th week (BGR-34)
c ($p<0.001$) Vs HbA1c at the 12th week (Sitagliptin Vs BGR-34)

**Random Blood Sugar (RBS)**

As depicted in Figure 2(A), the BGR-34 treatment for the period of 12 weeks produced a significant difference from the baseline RBS (250.32 mg/dl ± 5.645 mg/dl). The observed values of RBS reduced significantly in the study population over the period of 4 weeks, 8 weeks and 12 weeks which was 243.76 mg/dl ± 5.605 mg/dl, 217.24 mg/dl ± 5.426 mg/dl, and 114.4 mg/dl ± 2.596 mg/dl respectively. The study population receiving Sitagliptin also produced a similar effect as BGR-34, as the RBS value declined over the period of 12 weeks.

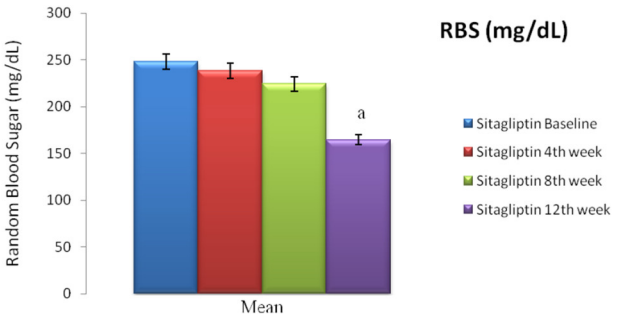
**Figure 2 (A).** Effect of BGR-34 on RBS over the period of 4, 8 and 12 weeks




a ($p<0.001$) Vs RBS at the 12th week BGR-34

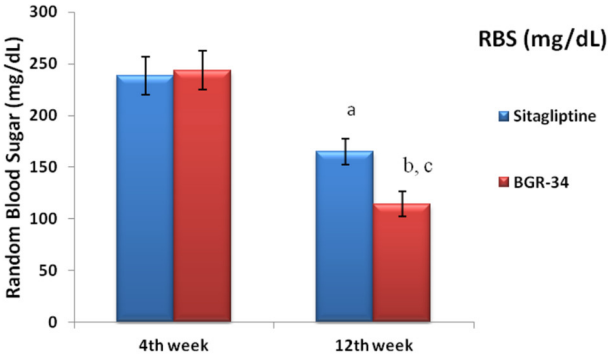
The values of RBS over the period of 4 weeks, 8 weeks and 12 weeks when compared to the baseline RBS (248.2 mg/dl ± 8.174 mg/dl) were observed as 238.46 mg/dl ± 7.947 mg/dl, 224.46 mg/dl ± 7.724 mg/dl, 164.66 mg/dl ± 5.327 mg/dl as depicted in Figure 2(B). As shown in Figure 2(C), in the comparative reduction of RBS in both study groups, it was observed that the group receiving BGR-34 had a statistically significant reduction in RBS when compared to the group receiving Sitagliptin ($P<0.001$) over the period of 12 weeks.

**Figure 2(B).** Effect of Sitagliptin on RBS over the period of 4, 8 and 12 weeks




a ($p<0.01$) Vs RBS at the 12th week Sitagliptin

**Figure 2(C).** Effect of BGR-34 and Sitagliptin after 4 and 12 weeks on Random Blood Sugar (RBS) values in type 2 diabetic patients.



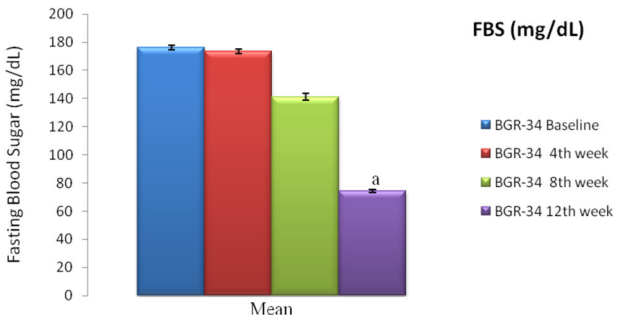

a ($p<0.05$) Vs RBS at the 4th week Vs 12th week (Sitagliptin)
b ($p<0.001$) Vs RBS at the 4th Vs 12th week (BGR-34)
c ($p<0.005$) Vs RBS at the 12th week (Sitagliptin Vs BGR-34)

**Fasting Blood Sugar**

The BGR-34 treatment for the period of 12 weeks produced a significant difference from the baseline FBS (176.4 mg/dl ± 1.685 mg/dl) as depicted in Figure 3(A). The observed values of FBS reduced significantly in the study population over the period of 4 weeks, 8 weeks and 12 weeks which were 173.3 mg/dl ± 1.607 mg/dl, 141.22 mg/dl ± 2.672 mg/dl, and 74.28 mg/dl ± 0.889 mg/dl respectively. The study population receiving Sitagliptin also produced a similar effect as BGR-34, as the FBS value declined over the period of 12 weeks. The values of FBS over the period of 4 weeks, 8 weeks and 12 weeks when compared to the baseline FBS (177.12 mg/dl ± 4.729 mg/dl) were observed as 165.4 mg/dl ± 4.367 mg/dl, 151.1 mg/dl ± 4.226 mg/dl, 151.1 mg/dl ± 4.226 mg/dl as depicted in Figure 3(B). As shown in Figure 3(C), in the comparative reduction of FBS in both study

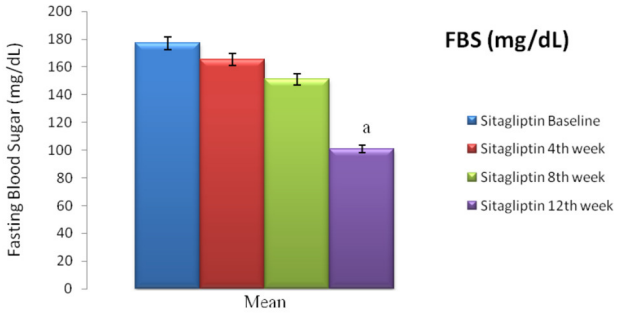
groups, it was observed that the group receiving BGR-34 had a statistically significant reduction in FBS when compared to the group receiving Sitagliptin (P<0.001) over the period of 12 weeks.

**Figure 3(A).** Effect of BGR-34 on FBS over the period of 4, 8 and 12 weeks



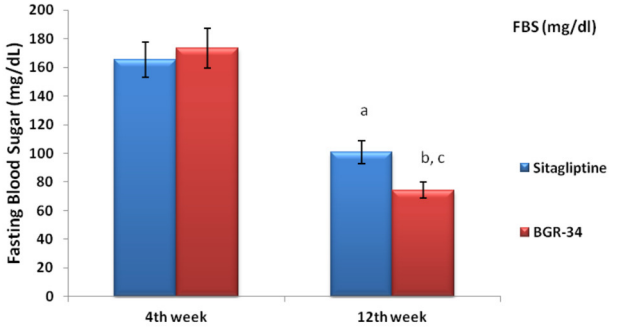

a (p<0.001) Vs FBS at the 12th week BGR-34

**Figure 3(B).** Effect of Sitagliptin on RBS over the period of 4, 8 and 12 weeks




a (p<0.005) Vs FBS at the 12th week Sitagliptin

**Figure 3(C).** Effect of BGR-34 and Sitagliptin after 4 and 12 weeks on Fasting Blood Sugar (FBS) values in type 2 diabetic patients.

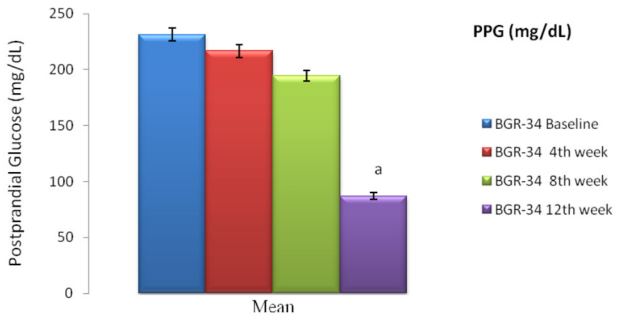



a (p<0.05) Vs FBS at the 4th week Vs the 12th week (Sitagliptin)
b (p<0.001) Vs FBS at the 12th week (BGR-34)
c (p<0.005) Vs FBS at the 12th week (Sitagliptin Vs BGR-34)

**Postprandial Glucose**

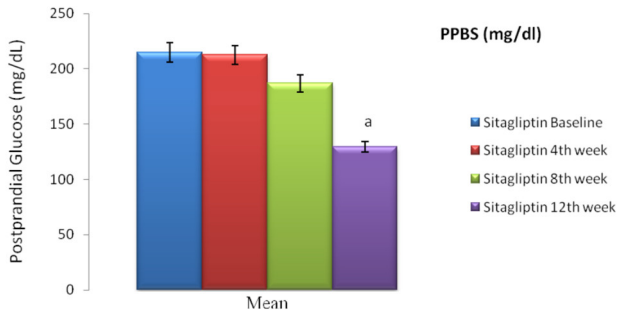
A significant change was observed in Post Prandial Blood Sugar (PPBS) in the patients receiving the BGR-34 treatment for the period of 4 weeks (216.84 mg/dl ± 5.773 mg/dl), 8 weeks (186.94 mg/dl ± 7.667 mg/dl) and 12 weeks (87 mg/dl ± 2.273 mg/dl) when compared to the baseline PPBS (231.38±5.78). A similar pattern was observed with Sitagliptin, as it was observed that PPBS declined significantly from 214.92 mg/dl ± 8.544 mg/dl to 212.58 mg/dl ± 8.478 mg/dl in 4 weeksand 8 weeks respectively and 129.54 mg/dl ± 4.725 mg/dl in the 12thweek(Figure 4 A, B). As shown in Figure 4(C), in the comparative reduction of PPBS in both study groups, it was observed that the group receiving BGR-34 had a statistically significant reduction in FBS when compared to the group receiving Sitagliptin (P<0.001) over the period of 12 weeks. The adverse effects were observed in 4% (2/50) of patients in the BGR-34 treated group and in 14% (7/50) of the patients in the Sitagliptin treated group. The patients receiving BGR-34 reported the gastric problem in 4% (2/50) whereas in the Sitagliptin treated group, the patients experienced the abdominal pain in 5% (*n* =1), nasophayngitis in 4% (*n* =2), GIT upset and constipation in 4% (*n*=2) nausea and diarrhoea were observed in two patients (4%). In the present study, to assess the safety of BGR-34 and Sitagliptin, the analyses of various biochemical parameters were done which revealed no significant changes in the patients. The treatment with Sitagliptin had a neutral effect on the body weight. However, in the Sitagliptin treated group, a slight increase was observed in the white blood cell count, primarily due to a small increase in the absolute neutrophil count and in the uric acid levels, but which were not significant. In addition to this, all other safety parameters viz. serum creatinine, the total leukocyte count, the differential leukocyte count, SGOT and SGPT, haemoglobin did not show any significant change. The present study confirms that the drugs were well tolerated and the patients showed full compliance toward the treatment during the study.

**Figure 4(A).** Effect of BGR-34 on PPBS over the period of 4, 8 and 12 weeks



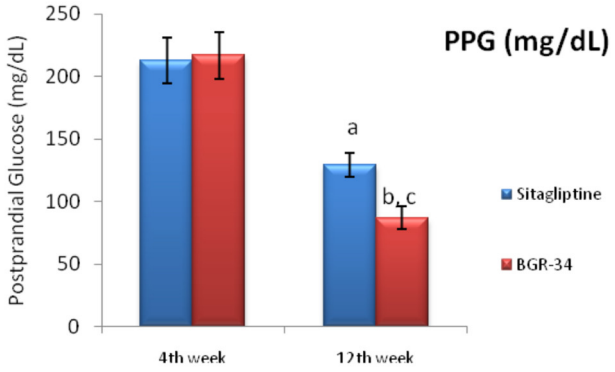

a (p<0.001) Vs PPG at the 12th week BGR-34

**Figure 4(B).** Effect of Sitagliptin on PPBS over the period of 4, 8 and 12 weeks




a ($p<0.001$) Vs PPG at the 12th week Sitagliptin

**Figure 4(C).** Effect of BGR-34 and Sitagliptin after 4 and 12 weeks on Post Prandial Blood Sugar (PPBS) values in type 2 diabetic patients.




a ($p<0.05$) Vs PPG at the 4th week Vs 12th week (Sitagliptin)
b ($p<0.001$) Vs PPG at the 12th week (BGR-34)
c ($p<0.05$) Vs PPG at the 12th week (Sitagliptin Vs BGR-34)

## DISCUSSION

Diabetes mellitus is a highly prevalent metabolic disorder that has affected nearly half of the population around the world. It is characterised with significantly high glucose levels in the blood which is associated with its typical clinical presentation of polyuria, polydipsia and polyphagia. Diabetes mellitus when left untreated may increase the likelihood of various metabolicchanges resulting invarious micro- and macro-vascular complications affectingthe eyes, kidney, heart and nerves (9). With availability of numerous oral therapeutic agents at the market, managing T2DM is still challenging and requires strict discipline in the diet and lifestyle. Numerous studies have been conducted implicating various therapeutic agents, currently available for the management of T2DM to assess their safety and efficacy. The available therapeutic modalities act either by increasing the secretion of insulin from pancreas or increasing the glucose uptake and decreasing gluconeogenesis, resulting in the reduced plasma glucose concentration (10). A regular and optimum control of the glucose levels can decrease the progression of the disease and further reduce the chances of occurrence of complications. The available oral hypoglycaemic drugs do not restore normal glucose homeostasis for a longer period and they are not free from side effects such as hypoglycemia, kidney diseases, GIT problems, hepatotoxicity, heart risk problems, insulinoma and they have to be taken for the rest of life (11). However, the treatment with herbal medications is trending nowadays. Various herbal drugs have also shown beneficial effects in controlling the blood glucose levels in the patients suffering from T2DM (10). Unfortunately, the role of herbal agents in the management of T2DM is still questionable due to the lack of clinical data on their safety and efficacy. Thus, the present study was conducted in order to generate the evidence regarding efficacy and safety of the herbal drug, "BGR-34" with the established oral hypoglycaemic agent "Sitagliptin" in the patients with the type 2 diabetes mellitus. Sitagliptin phosphate has already been the US FDA approved modality for the management of T2DM since 2006 (7). Sitagliptin, with already proven safety and efficacy profile in managing T2DM through several clinical studies, has an established market worldwide. Many double-blind studies were conducted in which Sitagliptin was compared to another DPP-4 inhibitor as a monotherapy or as an add-on therapy; with other oral hypoglycaemic drugs (12). Several doses of Sitagliptin were used to check the efficacy of the drug at various doses in trials lasting for 18-52 weeks (13). Sitagliptin given at doses of 100 mg and 200 mg was found to be significant (14) in reducing mild to moderate hyperglycemia; improving glycosylated haemoglobin; fasting plasma glucose; and the post prandial glucose levels. A similar study was conducted for 24 weeks, in which Sitagliptin significantly reduced HbA1c, fasting blood glucose, and post-prandial glucose ($p < 0.001$). In the same study, 100 mg and 200 mg of Sitagliptin were compared to the subjects receiving placebo, where 41% and 45% of the study population received Sitagliptin 100 mg and 200 mg respectively and only 17% of the study population received placebo. The result depicted a significant correlation between HbA1c (baseline) and the treatment with higher efficacy in the patients with higher baseline HbA1c (15). Furthermore, in the study, 100 mg dose of Sitagliptin was found to be well tolerated in the type 2 diabetic patients and provided an effective and sustained control of HbA1c, fasting glucose and post-prandial glucose. It was also found that Sitagliptin carries a lower risk of hypoglycaemia (16). Moreover, it has also been found that 100 mg dose of Sitagliptin once daily significantly lowers the glycemic level and β-cell functions of pancreas in the patients who had an inadequate control of the glycemic levels with Glimepiride or Glimepiride plus Metformin therapy. It is clear from most of the trails that sitagliptin produces the adequate improvements in the glycemic parameters. Apart from this, sitagliptin when given as a monotherapy lowers HbA1c levels up to 0.94%. However, when used in the combination with other oral hypoglycaemic drugs, it can provide an additional reduction in the glycemic levels in the body (17).

On the other hand, the preclinical study on BGR-34 has proven it to be safe and effective, with clinical studies

demonstrating 67% of success (NBRI). A doubleblind study for BGR-34 was conducted by the CSIR to check its efficacy in the patients with the type 2 diabetes mellitus at Aggarwal Hospital, New Delhi. There were, a total of 48 patients selected for the study consisting of 30 males and 18 females after applying the inclusion as well as exclusion criteria. It was conducted for a period of 16 weeks. The patients were divided into two groups in which 24 patients were on the BGR-34 therapy and other 24 patients were in the placebo group. The biochemical results revealed that highly significant results were obtained in 15 patients receiving BGR-34. There were relatively lower levels of blood sugar. However, there was no reduction in glycosylated haemoglobin in the patients receiving placebo. Instead, a significant increase was observed in the subjects receiving BGR-34 during a 16week study. The present study was a randomized, parallel, comparative study in which the subjects were randomly selected. The outcome of the study revealed that there was a significant reduction in HbA1c, Random Blood Sugar (RBS), Fasting Blood Sugar (FBS) and Postprandial Glucose (PPG) with BGR-34 when compared to Sitagliptin. BGR-34 acts through several mechanisms and maintains the optimum blood glucose levels, relieves the symptoms associated with T2DM and reduces the chances of complications and imparts a good quality of life in the patients with high blood sugar levels. The possible reason for better efficacy could be due to its several nutritive phytoconstituents and antioxidants which protect β-cells from damage, promote the repair and regeneration of β-cells. Thus, the insulin production is increased by improving the functional capacity of β-cells.

## CONCLUSION

Based on the results obtained in the present study, it can be concluded that BGR-34 is effective in reducing high blood sugar levels and is more potent and efficacious in decreasing the glycemic levels possibly by modulating the insulin release and strengthening the β-cell functional capacity. It also exerts a powerful anti-oxidant action which prevents the development of diabetic complications. This reflects that the BGR-34 therapy is more effective drug in the treatment of diabetes suggesting that it is better in efficacy, reliability and affordability with little or no adverse effects. From the present study, it has emerged that this treatment favourably contributes to the health effective benefits by inhibiting the disease progression and fulfilling the alternate goals of the management of diabetes mellitus. Strict glycaemic control favours the goals of managing diabetes mellitus.

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## CONFLICT OF INTERESTS

There are no conflicts of interest.

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Nil.

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# NUTRITIONAL STATUS DISORDERS IN STUDENT POPULATION

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# POREMEĆAJ STATUSA UHRANJENOSTI U STUDENTSKOJ POPULACIJI

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## ABSTRACT

*Obesity is a global health problem associated with numerous pathological conditions. Unhealthy eating habits and the lack of regular physical activity are considered the most common cause of disordered nutritional status. The aim of the research was to determine the nutritional status in student population and the predictors which determine this condition. The cross-sectional study was conducted on 262 students of the Faculty of Medical Sciences in Kragujevac (130 males and 132 females). Body weight and height, body mass index (BMI) and visceral fat (VF) were measured. Each respondent completed a specially designed questionnaire considering sociodemographic data, eating habits and physical activity. The majority of students have normal BMI values (75.6%), 5.3% were classified as underweight, 14.9% as overweight and 4.3% as obese. Normal VF values were found in 93.1% of subjects, while high in 5.7% and very high in 1.1%. A statistically significant difference in BMI and VF values was found between male and female gender (24.41 vs. 21.05, Sig = 0.000 and 5.47 vs. 3.07, Sig = 0.000, respectively), as well as between students of the first 4 and the last 2 years of study (Sig = 0.019 and 0.000 respectively). Unhealthy eating habits, such as the consumption of sweets, snacks, fast foods and white bread, and the absence of regular physical activity were statistically more present in overweight/obese respondents. Given the significant presence of pre-obesity/obesity in the examined population, corrective measures should be taken in this population in order to avoid a major health problem in the future.*

**Keywords**: *Obesity, nutrition, student population*

## SAŽETAK

*Gojaznost predstavlja globalni zdravstveni problem, povezan sa brojnim patološkim stanjima. Nezdrave navike u ishrani i nedostatak redovne fizičke aktivnosti smatraju se najčešćim uzrokom gojaznosti. Cilj istraživanja bio je utvrditi nutritivni status studentske populacije i prediktore koji na njega utiču. Studija preseka sprovedena je na 262 studenta Fakulteta medicinskih nauka u Kragujevcu (130 muškog i 132 ženskog pola), podeljenih u 6 uzrasnih kategorija, shodno godini studija. Ispitanicima su mereni telesna težina i visina, indeks telesne mase (ITM) i zastupljenost visceralnih masti (VM). Svaki ispitanik je popunio specijalno dizajnirani upitnik koji se ticao sociodemografskih podataka, navika u ishrani i fizičke aktivnosti. Većina studenata je imala normalne vrednosti ITM (75,6%), dok je 5,3% klasifikovano kao pothranjeno i 14,9% kao predgojazno, a 4,3% kao gojazno. Normalne vrednosti VM nađene su u 93,1% ispitanika, visoke u 5,7%, a veoma visoke u 1,1%. Statistički značajna razlika u vrednostima ITM i VM nađena je između muškog i ženskog pola (24,41 vs. 21,05, Sig = 0,000; 5,47 vs. 3,07, Sig=0,000), kao i između studenata prve 4 i poslednje 2 godine studija (Sig=0,019, odnosno 0,000). Nezdrave navike u ishrani, poput konzumacije slatkiša, grickalica, brze hrane i belog hleba, kao i odsustvo redovne fizičke aktivnosti, statistički značajno su bili prisutniji među predgojaznim/gojaznim ispitanicima. Obzirom na značajno prisutvo predgojaznosti/gojaznosti u ispitivanoj populaciji, potrebno je preduzeti korektivne mere u ovoj populaciji, kako bi se izbegao veliki zdravstveni problem u budućnosti.*

**Ključne reči:** *gojaznost, ishrana, studentska populacija*

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## INTRODUCTION

Proper nutrition and regular physical activity are one of the basic prerequisites for a healthy life. The basic principles of healthy nutrition are moderation, meal regularity and variety in choice of nutrients (expressed through the World Health Organization ("WHO") pyramid), with regular physical activity. Violation of these principles over a longer period of time leads to nutritional status disorders, in the direction of malnutrition or obesity, and, consequently, to potential health disorders. Obesity, a nutritional status disorder significantly more common than malnutrition, is often defined "as an abnormal or excessive fat accumulation in adipose tissue, to the extent that health may be impaired" (1, 2).

In addition to energy imbalance, there are many other reasons that can lead to the development of obesity, such as physiological (pregnancy, menopause), pathological (chromosomal disorders, metabolic diseases, gestational diabetes, some tumors, etc.) and medicamentous (antipsychotics, antidepressants, antiepileptics, etc.) (1-4) are.

The great importance of obesity lies in its prevalence, which is increasing worldwide in alarming rate, in both developed and underdeveloped countries (4). Since 1980 worldwide obesity has more than doubled. In 2014 study more than 1.9 billion adults (39% of global population) were overweight. Of these over 600 million (13% of global population) were obese. In 2014 more than 41 million children under the age of 5 were overweight or obese (3). In study conducted in Serbia in 2006, 55.7% of population was overweight with 19% being obese (5).

The fact that worries is the increase of obesity in the category of young adults and students. Two studies conducted on USA college students have shown that 3 of 10 college students were overweight or obese (6, 7). Aside from America, the high prevalence of obesity in young adults is also present in other countries around the world, more common in male subgroup (8-12). Although it would be expected that obesity is not so much present among medical students, due to better knowledge in terms of harmfulness and consequences of obesity, studies have shown that even this population of students is not an exception, with overweight also being more present in male population (13-17). Obesity is a serious health problem of modern age. The reasons are, beside its omnipresence, serious complications and diseases associated with obesity. It has been shown that obesity is an important risk factor for many diseases: cardiovascular (hypertension, acute coronary syndrome, stroke), respiratory (obstructive sleep apnea), gastrointestinal (fatty liver, gallbladder, gout), metabolic and endocrine disorders (diabetes mellitus type 2, hypercholesterolemia, metabolic syndrome X), some types of carcinoma, negligible and underestimated psychological and self-esteem disorders and many others (2-4, 18, 19). In addition to this, Blackburn GL et al. study has shown that obesity is related with premature mortality (20). For these reasons, adequate classification of obesity is of great importance because it would help us to timely detect obesity, degree of risk and, consequently, give us a better chance to prevent comorbidities. Although there are several obesity classifications, the most commonly used is the one based on Body Mass Index (BMI), proposed by the WHO. Given the ease of performance and satisfactory reliability, BMI can be used as a predictor of the population and large groups (3).

## THE AIM OF THE PAPER

The aim of the research was to determine the nutritional status in student population and the predictors which determine this condition.

## PATIENTS AND METHODS

### Sample

The study was conducted on students of medicine at the Faculty of Medical Sciences in Kragujevac, from September to October 2016, as a descriptive cross-sectional study. The study involved 262 students (130 male and 132 female) from all 6 years of study, with approximately equal gender distribution in each category: 1$^{st}$ year - 30 (14; 16); 2$^{nd}$ year - 47 (23; 24); 3$^{rd}$ year - 37 (19; 18); 4$^{th}$ year - 49 (25; 24); 5$^{th}$ year - 51 (26.25); 6$^{th}$ year - 48 (23; 25). The minimum number of respondents calculated by G-Power software for $\alpha$ err prob = 0.05; power (1-$\beta$ err prob) = 0.95; was 220. Excluding factors were: pregnancy, spine deformities and conditions that are reliably known to be a limitation for using BMI as a tool of assessing nutritional status.

### Measurements

All students were measured:

1. Body height;

2. Body weight;

3. Body Mass Index (BMI);

4. Visceral fats;

1. Body height was measured to the nearest 0.1cm with standard stadiometer.

For items 2-4, the Omron BF511 was used. The device calculated BMI using the entered height and measured weight (measured to the nearest 0.1kg) by the standard outlet BMI formula: Weight[kg]/Squared Height[m$^2$]. Visceral fats were measured by the bioelectrical impedance method. In order to reduce the possibility of false results, the recommendations were followed by the manufacturers - respondents who were measured barefoot and light clothing. They did not eat 3 hours and did not drink 1 hour before the measurement.

Based on the obtained results, students were classified according to the nutritional status categories, using the already established categorization tables, as following:

1. For BMI:

Underweight - BMI<18.5kg/$m^2$
Normal weight - BMI 18.5-24.9 kg/$m^2$
Overweight/Pre obese - BMI of 25-29.9 kg/$m^2$
Obesity class 1 - BMI 30-34.9 kg/$m^2$
Obesity class 2 - BMI 35-39.9 kg/$m^2$
Obesity class 3 - BMI ≥40 kg/$m^2$;

2. For Visceral Fat (cut point given by the manufacturer):

a) Normal: 1-9

b) High: 9-14

c) Very High: >15

**Food Questionnaire**

On 262 respondents, 258 adequately filled out a questionnaire (which is a part of the WHO Food Frequency Questionnaire) regarding sociodemographic data, daily activities and eating habits, in terms of its quantity and quality.

For the purpose of research, eating habits have been observed in relation to basic principles of proper nutrition according to the WHO pyramid of nutrition, as following:

1. Cereals - Healthy use of cereals implied the participation of cereals with 30-45% in total daily calorie intake, mainly grains with full grain and preserved biological value (for example black and integral flour). Deviations from these recommendations were considered an unhealthy diet (for example significantly lower or higher consumption, the use of white flour, etc.)

2. Fruits - Healthy use of fruit implied everyday use with participation in total daily caloric intake of 10-15%

3. Vegetables - Healthy use of vegetables implied everyday use with participation in total daily caloric intake of 10-15%

4. Milk and milk products - Healthy use of milk and milk products implied everyday use with participation in total daily calorie intake of 10%, mainly low-fat products such as low-fat yogurt, kefir, non-fat cheese. Deviations from these recommendations were considered an unhealthy diet (for example lower or higher consumption, use of high-fat foods such as sour cream, fatty cheeses, butter, etc.)

5. Meat and meat products - Healthy use involved meat consumption 2 to 3 times a week, mainly low-fat/high-protein meat (such as chicken, fish, etc.). Deviations from these recommendations were considered as unhealthy diet (for example significantly reduced or more frequent use of meat, the consumption of high-fat meat such as pork, dried and cured meat, fast-food, etc.)

6. Visible Fats, Sugars and Sweets: Proper nutrition involved foods from this category up to the total daily calorie intake of 5%. Deviations from recommendations were considered unhealthy diet (for example frequent use of sweets, snacks, fried foods).

At the same time, lifestyle habits were evaluated considering physical activity and time spent on TV/PC (criteria for unhealthy habit was more than 3h a day).

**Statistical analysis**

The data was statistically analyzed using statistical software package SPSS (ver. 21). The results are given as mean ±SE, where SE is standard error of the mean. The Kolmogorov-Smirnov test was used to determine the normality of distribution. Values of nutritional status parameters were compared using the Mann-Whitney test, while categories of nutritional status parameters were compared using the Chi-square test. Chi-square and Fisher's Exact Tests were used to compare eating and lifestyle habits to nutritional status categories. These two are also used to compare habits between age and gender categories. Values of Sig<0.05 were taken to be statistically significant.

## RESULTS

Table 1 gives the examined parameters of the nutritional status of the global sample: the majority of students have normal BMI values (75.6%), 5.3% were classified as underweight, 14.9% as overweight, and 4.3% as obese (by classes of obesity: 3,1%, 0.8%, and 0.4%, respectively). Normal levels of visceral fats were found in 93.1% of respondents, while high in 5.7% and very high in 1.1%. Table 2 shows the average values of BMI and Visceral Fats within age and gender categories. There is a statistically significant difference in BMI and VF values between men and women, found on the global sample (24.41 vs. 21.05, Sig=0.000 and 5.47 vs. 3.07, Sig=0.000, respectively). Statistically significant difference in BMI and VF values between the students of the first 4 and the last 2 years of study was found on the global sample (Sig = 0.019 and 0.000, respectively) and within the male population (Sig = 0.007 and 0.000, respectively), while such a difference within the female population was found only for VF parameter (Sig=0.036), not for BMI. Also, we notice that average male student of 6th year is actually overweight according to BMI classification. Tables 3 and 4 show the prevalence of healthy and unhealthy eating, as well as lifestyle habits (according to WHO's food pyramid recommendations) measured by BMI and VF categories. We notice that significant number of students in total, not just regarding presence of obesity, has unhealthy habits when it comes to consumption of cereals, milk and milk products, meat and meat products, sweets, preparing meals and physical activity. Chi-square test showed statistically significant impact on high levels of BMI (>25) and VF for unhealthy habits considering: consumption of cereals (0.016; 0.029), meat and meat products (0.000; 0.027), fast food (0.004; /), sweets (0.001; /), snacks (0.002; /), fruits (/; 0.025), preparing meals (0.001; 0.049), less frequent physical activity (0.001; 0.000; 0.001)

and more frequent physical inactivity observed as time spent on TV/PC (0.000; 0.000).

**Table 1.** Percentage distribution by nutritional status parameters categories

| BMI | | | VF | |
|---|---|---|---|---|
| Underweight | | 14 (5.3%) | Low | 244 (93.1%) |
| Normal weight | | 198 (75.6%) | High | 15 (5.7%) |
| Overweight | | 39 (14.9%) | Very High | 3 (1.1%) |
| Obesity | Class 1 | 8 (3.1%) | | |
| | Class 2 | 2 (0.8%) | | |
| | Class 3 | 1 (0.4%) | | |

**Table 2.** Average values of nutritional status parameters by age and gender categories (SD)

| Year of study | Gender | BMI | VF |
|---|---|---|---|
| **1 st Year** | Male | 23.18 (1.59) | 4.00 (1.61) |
| | Female | 21.29 (2.48) | 3.00 (1.32) |
| | Both sexes | 22.17 (2.29) | 3.47 (1.50) |
| **2 nd Year** | Male | 23.22 (2.12) | 4.39 (1.90) |
| | Female | 21.11 (2.44) | 2.83 (1.09) |
| | Both sexes | 22.14 (2.50) | 3.6 (1.7) |
| **3 rd Year** | Male | 24.25 (3.27) | 5.68 (3.04) |
| | Female | 20.22 (2.20) | 2.83 (0.92) |
| | Both sexes | 22.23 (3.43) | 4.3 (2.7) |
| **4 th Year** | Male | 24.31 (4.33) | 5.56(3.65) |
| | Female | 20.67 (3.11) | 2.92(1.25) |
| | Both sexes | 22.53 (4.17) | 4.27 (3.00) |
| **5 th Year** | Male | 24.85(3.82) | 6.35 (3.51) |
| | Female | 21.36 (2.40) | 3.24 (1.05) |
| | Both sexes | 23.14 (3.6) | 4.82 (3.00) |
| **6th Year** | Male | 26.11 (3.66) | 7.70 (3.34) |
| | Female | 21.54 (2.50) | 3.48 (1.66) |
| | Both sexes | 23.73 (2.84) | 5.5 (3.3) |
| **Total** | Male | 24.41 (3.47) | 20.15 (6.43) |
| | Female | 21.05 (2.54) | 28.47 (6.96) |
| | Both sexes | 22.73 (3.47) | 24.34 (7.85) |

**Table 3**. Presence of healthy and unhealthy eating and lifestyle habbits by BMI categories

| Food | | Underweight | Normal Weight | Overweight | Obesity | | | Overall |
|---|---|---|---|---|---|---|---|---|
| | | | | | Class 1 | Class 2 | Class 3 | |
| Cereals | Unhealty | 9 | 128 | 31 | 8 | 2 | 1 | 179 |
| | Healthy | 5 | 68 | 8 | 0 | 0 | 0 | 81 |
| Fruits | Unhealty | 1 | 24 | 6 | 3 | 0 | 1 | 35 |
| | Healthy | 13 | 170 | 33 | 5 | 2 | 0 | 223 |
| Vegetables | Unhealty | 0 | 21 | 1 | 2 | 0 | 0 | 24 |
| | Healthy | 14 | 173 | 38 | 6 | 2 | 1 | 234 |
| Milk and products | Unhealty | 6 | 122 | 26 | 5 | 0 | 0 | 159 |
| | Healthy | 8 | 74 | 13 | 3 | 1 | 1 | 100 |
| Meat and products | Unhealty | 8 | 139 | 37 | 8 | 2 | 1 | 195 |
| | Healthy | 6 | 55 | 1 | 0 | 0 | 0 | 62 |
| Sweets | Unhealty | 5 | 77 | 26 | 4 | 1 | 1 | 108 |
| | Healthy | 9 | 123 | 13 | 4 | 1 | 0 | 150 |
| Snacks | Unhealty | 3 | 18 | 11 | 1 | 2 | 0 | 35 |
| | Healthy | 11 | 186 | 28 | 7 | 0 | 1 | 223 |
| Preparing meals | Unhealty | 5 | 71 | 25 | 5 | 2 | 0 | 108 |
| | Healthy | 9 | 123 | 14 | 3 | 0 | 1 | 150 |
| Physical activity | Unhealty | 7 | 110 | 30 | 8 | 2 | 1 | 158 |
| | Healthy | 7 | 84 | 9 | 0 | 0 | 0 | 100 |
| TV/PC | Unhealty | 0 | 27 | 14 | 5 | 1 | 1 | 48 |
| | Healthy | 14 | 167 | 25 | 3 | 1 | 0 | 210 |

**Table 4.** Presence of healthy and unhealthy eating and lifestyle habbits by Visceral Fat categories

| Food | | Normal | High | Very High | Overall |
|---|---|---|---|---|---|
| Cereals | Unhealty | 160 | 14 | 3 | 177 |
| | Healthy | 80 | 1 | 0 | 81 |
| Fruits | Unhealty | 28 | 5 | 1 | 34 |
| | Healthy | 210 | 10 | 2 | 222 |
| Vegetables | Unhealty | 22 | 2 | 0 | 24 |
| | Healthy | 216 | 13 | 3 | 232 |
| Milk and products | Unhealty | 146 | 12 | 0 | 158 |
| | Healthy | 94 | 3 | 2 | 99 |
| Meat and products | Unhealty | 175 | 15 | 3 | 193 |
| | Healthy | 62 | 0 | 0 | 62 |
| Sweets | Unhealty | 96 | 9 | 2 | 107 |
| | Healthy | 142 | 6 | 1 | 149 |
| Snacks | Unhealty | 30 | 2 | 2 | 34 |
| | Healthy | 208 | 13 | 1 | 222 |
| Preparing meals | Unhealty | 95 | 10 | 2 | 107 |
| | Healthy | 143 | 5 | 1 | 149 |
| Physical activity | Unhealty | 138 | 15 | 3 | 156 |
| | Healthy | 100 | 0 | 0 | 100 |
| TV/PC | Unhealty | 38 | 7 | 2 | 47 |
| | Healthy | 200 | 8 | 1 | 209 |

## DISCUSSION

The main importance of the study lies in the proven correlation between the nutritional status disorders (especially obesity) and numerous comorbidities, which in the first place cardiovascular etiology (2-4, 18, 19). The significance of the study is also due to the fact that, despite the importance and actuality of the topic, similar study on the student population in Serbia has not yet been published, at least known to the authors.

Majority of the students had normal BMI values (75.6%), 5.3% were classified as underweight, 14.9% as overweight, and 4.3% as obese (by classes of obesity: 3.1%, 0.8% and 0.4%, respectively). Although the prevalence of overweight and obesity was, as expected, notably lower than the research of Grujić V. et al. conducted on adult population in Serbia, where 55.7% of subjects were overweight and 19% obese, the results of our study are far from satisfactory (5).

High prevalence of pre-obesity and obesity in the student population is not only present in our country. Methodologically similar studies on larger samples have shown a similar

or greater prevalence of nutritional status disorders in the countries of North America, the European Union, Africa and Asia (6, 7, 9, 21-24, 33). The high prevalence of overweight and obesity in socioeconomic and culturally different countries asserts the global nature of this problem. The only exception were the countries of East and Southeast Asia (8, 25). Although medical students would be expected to have healthier eating habits and lower prevalence of obesity, due to better knowledge of obesity and its consequences, results of the studies show the opposite (13, 15-17, 26-28). A potential explanation was given by Wirginia Likus et al in a study conducted on medical students in Poland, that showed a very limited awareness of medical students about the effects of obesity on health. On the other hand, due to the scope of learning, students are generally forced to spend most of their time sedentary, deprived of time for regular physical activity and regular, healthy rest and sleep (29). Besides, in the medical student population Abbate C et al. showed the high prevalence of overweight/obesity in adult healthcare workers in general, probably due to the stressfulness of their jobs (30).

A significant disparity in terms of nutritional status parameters was found between male and female gender, with average values for BMI being 24.41 and 21.05. The disparity of visceral fats was 5.47 and 3.07 with statistically significant difference for both examined parameters. A significant difference in the nutritional status among genders has been shown in many other studies, both in student and adult population (5, 9, 13, 16, 21, 23, 24, 25, 30), not related to prevalence of overweight/obesity in the examined population in general. A potential explanation was given in the Margareta et al. study, which showed that women more often, and more precisely, consider themselves pre obese/obese, and would rather have some kind of diet (21). Also, the reason for disparity in the nutritional status among genders could be an imposed methodological deficiency, observed during data collection. It could be that overweight/obese female students have avoided public measurement, while this was not the case with male students.

A statistically significant difference in nutritional status was also found among younger and older students (observed through students of the first 4 years and the last 2 years of studies). The difference was found for BMI on global sample and in male subgroup, and for VF on global sample and in both gender categories.

In the Gropper S et al. study, respondents were tracked from the first to the final year of study in terms of nutritional status parameters. In all examined categories, a statistically significant increase was observed (32). A possible explanation of this phenomenon is that, over time, the cumulative effects of sedentary and unhealthy lifestyle have appeared (31). Also, the increase in education level increases the prevalence of obesity (30). This fact could, more likely, explain the high prevalence of obesity in the medical student population in general, while its explanation of increase in obesity through the course of faculty education is debatable.

However, even here, the imposed methodological defect could be the cause of a great difference. While collecting data, it was noticed that younger students were not relaxed enough and that those who were pre obese/obese were avoiding measuring, while this was not the case with older students. Therefore, the similar methodological error could lead to a significant difference in the nutritional status parameters among age and gender categories. However, even if this is true and this effect is significant, this would only mean that the real prevalence of the nutritional status disorders in the examined population is greater than shown.

Examining the nutritional habits, we note that the influence of improper nutrition (according to the WHO criteria) and lack of physical activity on the development of nutritional status disorders are statistically significant. However, improper nutrition has long been associated with the development of pre-obesity and obesity. What worries more is the significant presence of unhealthy eating and lifestyle habits in young adults in general. Surveys that studied this topic point to the global character of this problem (9, 13, 15, 16, 26, 27, 33).

This may provide answers to many questions, imposed by this study results. First of all, this could be the reason for the high prevalence of pre-obesity/obesity in the student population in general. Also, it could explain the low prevalence of nutritionl status disorders in the countries of East and Southeast Asia, where studies on this topic have shown significantly healthier eating and lifestyle habits among young adults (8, 32). Secondly, the studies showed significantly healthier habits considering nutrition and physical activity among female, compared with male gender in majority of studies, which could explain the differences in these categories. And finally, the possible explanation of higher values of nutritional status parameters in senior students may be a cumulative effect of unhealthy eating habits which resulted in nutritional status disorders.

## CONCLUSION

Considering the extent of unhealthy habits among students in present, and low amount of resting time and stressfulness of jobs in the future, perhaps the examined population could reach, or even exceed the numbers of 55.7% and 19% in adulthood. Therefore, an immediate action for prevention of obesity and obesity-related health problems is needed in this population, in order to prevent a major health problem in the future.

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# ANALYSIS OF EXTERNAL ROOT RESORPTION OF THE SECOND MOLAR ASSOCIATED WITH AN IMPACTION OF THE THIRD MOLAR BY THE APPLICATION OF CBCT

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# ANALIZA EKSTERNE RESORPCIJE KORENOVA DRUGOG MOLARA POVEZANE SA IMPAKCIJOM TREĆEG MOLARA PRIMENOM CBCT-A

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## ABSTRACT

*External root resorption (ERR) of a tooth represents a process in which irreversible damage occurs to solid dental tissues, more precisely of the cement and dentin. External resorption occurs on the root surface or periodontal ligament, and it is manifested by the loss of cement and/or dentine. The prevalence of ERR of the second molar associated with an impacted third molar, based on retro-alveolar radiography and orthopantomography, ranges from 0.3% to 24.2%. The aim of this research was to analyze ERR of the upper and lower jaw associated with the impacted third molar by the application of the Come Beam Computed Tomography. This study included 96 third molars in the upper and lower jaw of a total of 46 analyzed patients' images. Total prevalence of the second molar ERR associated with the impacted third molar was 8.82%. The second molar ERR was observed in 8 patients (7.84%). Frequency of the second molar ERR in the lower jaw is higher, and it is 8.2%, while in the upper jaw it is only 1% (r = 0.032). Impacted third molars associated with the second molar ERR were most frequently in horizontal (5.1%) and mesioangular (4.1%) position (p = 0.000). The second molar ERR could be avoided by the preventive extraction of mesioangular or horizontal third molars. An adequate decision on the extraction of impacted third molars must be made by the careful assessment of risks and benefits of this surgical intervention. Before the surgical intervention, an adequate care of the second molar, adequate restoration, endodontic treatment or root resection should be done if necessary.*

**Keywords**: *CBCT, external resorption, impacted third molar, second molar*

## SAŽETAK

*Resorpcija korenova zuba predstavlja proces u kom dolazi do ireverzibinog oštećenja čvrstih zubnih tkiva, tačnije cementa i dentina. Eksterna resorpcija se odvija na površini korena, odnosno periodontalnog ligamenta i manifestuje se gubitkom cementa i / ili dentina. Prevalenca eksterne resopcije korenova drugog molara povezana sa impaktiranim trećim molarom, bazirana na retroalveolarnoj radiografiji i ortopantomografiji je u rasponu od 0,3% do 24,2%. Cilj ovog rada bila je analiza eksternih resorpcija korenova drugih molara gornje i donje vilice povezane sa impakcijom trećeg molara, primenom kompijuterizovane tomografije konusnog zraka (engl. Cone Beam Computer Tomography / CBCT). Ova studija je obuhvatila 96 trećih molara u gornjoj i donjoj vilici od ukupno 46 analiziranih snimaka pacijenata. Ukupna prevalenca eksterne resorpcije korenova drugog molara povezane sa impaktiranim trećim molarom iznosila je 8,82%. Eksterna resorpcija korenova drugog molara zabeležena je kod 8 pacijenata (7,84%). Zabeležena je veća učestalost eksterne resorpcije korenova drugog molara u donjoj vilici i iznosi 8,2%, dok je u gornjoj vilici samo 1% (p =0.032). Impaktirani treći molari povezani sa eksternim resorpcijama korenova drugog molara, najčešće su bili u horizontalnom (5,1%) i mezioangularnom (4,1%) položaju (p=0.000). Eksterne resorpcije korenova drugog molara bi se mogla izbeći preventivnom ekstrakcijom mezioangularno ili horizontalno postavljenih trećih molara. Adekvatna odluka o ekstrakciji impaktiranih trećih molara mora biti doneta pažljivom procenom rizika i prednosti koje donosi ta hirurška intervencija. Pre hirurške intervencije potrebno je adekvatno zbrinjavanje drugog molara, adekvatnom restauracijom, endodontskim tretmanom ili resekcijom korena ukoliko je to neophodno.*

**Ključne reči:** *CBCT, eksterna resorpcija, impaktirani treći molar, drugi molar*

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## INTRODUCTION

A root resorption can be a physiological or pathological process, which is characterized by irreversible damage to solid dental tissues like cement and dentin (1). Physiological resorption is a desirable process that occurs in the deciduous dentition, followed by the eruption of permanent substitutes (2). All root resorption of permanent teeth can be classified as pathological resorption (3). Resorption localization in relation to the affected root surface can be an internal and external (4). The internal resorption starts in pulp and it is a progressive destruction of intra-radicular dentine and dentinal tubule along the walls of the middle and apical third of a root canal (5). Cause of the internal resorption lies in infection, traumatic injury or orthodontic therapy (5). The external resorption occurs on the root surface or periodontal ligament and it is manifested by the loss of cement and/or dentin (6). A cause of the external root resorption (ERR) may be local factors (dental impaction, orthodontic treatment, cysts, tumors, periapical inflammatory conditions, luxation or reimplantation of the teeth, periodontal disease and teeth whitening) and systemic diseases (hypoparathyroidism, hyperparathyroidism, Morbus Paget, Tarner syndrome, Gaucher disease) (4).

Third molars that fail to achieve a functional position may have a pathological effect on adjacent tissues, including roots of the second molar. Presence of ERR and dental caries on the second molar's distal surface can be associated with the impacted adjacent third molar (7). This type of resorption often belongs to the group of aseptic resorptions and it occurs at the contact spot of the second and the impacted third molar (8). It is initiated by the pressure of the impacted third molar (9). The second molar ERR pathogenesis is related to the third molar impaction but still remains unknown (10). The opinion is that an injury of the periodontal membrane makes the inflammation which impacts reduced epithelium of an impacted tooth (10). The secretion of inflammatory mediators by reduced tooth epithelium contributes to the activity of osteoclasts and resorption of mineralized tissue (10).

The third molar eruption time is most commonly between the ages of 17 and 21 (7). Making a decision on the prophylactic removal of third molars without symptoms is not easy, for it is necessary to be acquainted with pathological conditions that can be related to third molars (11). The diagnosis of ERR is difficult to establish if the inflammation in the periapical tissue or pulpitis is not observed (7).

An accurate and timely diagnosis of ERR depends on different radiographic methods (12). Inspection and histological analysis are usually not possible (12). Prevalence of the second molar ERR associated with the impacted third molar, based on the retro-alveolar radiography and orthopantomography, ranges from 0.3% to 24.2% (8). Two-dimensional display, unequal magnification, image distortion and superposition affect their low diagnostic value (12, 13). The advantage of the Cone Beam Computerized Tomography (CBCT) is a high spatial resolution and a large number of multiplane images that do not overlap (12). Exposure to X rays is higher in CBCT (45 μSv - 477 μSv), which, in addition to high costs, reduces the routine use for diagnostic purposes (13).

## THE AIM OF THE PAPER

The aim of this research was to analyze ERR of the upper and lower jaw associated with the impacted third molar by the application of CBCT.

## PATIENTS AND METHODS

In this retrospective study, a total of 186 CBCT scans of patients treated in the Institute of Dentistry at the Faculty of Medical Sciences, the University of Kragujevac were examined. Images of patients who were subjected to CBCT in order to get a diagnosis (endodontics, prosthetics, orthodontics, implantology and oral surgery) were analyzed in the period starting from October 2014 to February 2019. For this study we have an approval of the Faculty of Medical Sciences, the University of Kragujevac and the Ethics committee (01-8190).

The Orthophos XG 3D (Sirona Dental Systems GmbH, Bensheim, Germany) was used to create images with a field size of 8X8cm. The GALAXIS v1.9.4 software (Sirona Dental Systems GmbH, Bensheim, Germany) was used for the three-dimensional reconstruction of images, with a high image definition (100 μm). All recordings were screened in a dimmed room on the Philips LED monitor with a size of 23 inches and a resolution of 1920 x 1080 pixels.

Criteria for an inclusion were recordings of patients older than 18 years, who had at least one-third of the molar and root development of more than two-thirds. Scans showing the existence of carious lesions and fixed prosthodontics on the second or third molar, pathological processes, tumors, bone defects or cysts in the molar region, as well as scans with incomplete third molars, were not analyzed. We included 46 patients' images with a total of 96 third molars in both jaws for the final treatment of results.

ERR can be localized in the apical, middle or cervical third of root (8). In relation to the amount of the lost dental tissue, we have a slight, moderate and severe resorption. The slight resorption is when less than a half of dentin tissue is resorbed. The moderate resorption is when at least a half of dentin tissue is resorbed but not in contact with the dental pulp, and severe resorption is when there is a contact with dental pulp (8).

In order to determine the third molar position and inclinations, we used the Winter and Pell-Gregory classifications (14, 15). Based on the Winter classification, the third molar is mesioangulary positioned when an angle between the longitudinal axis and occlusal surface of the second molar is between 31 and 60 degrees. If that value is less than 31 degrees a tooth is positioned horizontally and vertically if it is greater

than 60 degrees (9). The distoangular position defines a distally oriented longitudinal axis of the third molar in relation to a longitudinal axis of the second molar. Based on the Pell-Gregory classification, vertical position of the third molar can be at the occlusal level (class A), between the occlusal surface and cement-enamel boundary (class B); and below the level of cement-enamel boundary (class C) of the second molar (9).

Statistical processing of the collected data was done by the application of SPSS v20.0 software (SPSS Inc., Chicago, IL, USA). Average values and standard deviations of the measured parameters were calculated and descriptive statistics were made. The accuracy of data was calculated by the Shapiro-Wilk test. For the numerical data that followed a normal distribution, we used the ANOVA test, and for data that did not follow a normal distribution we used the Mann-Whitney test. The chi-square test was used for categorical variables.

## RESULTS

This study included 96 third molars in the upper and lower jaw of 46 analyzed patients' images. An average value of patients' years of age was 38.3 (ranging from 19 to 75), 58.7% male, and 41.3% female.

Prevalence of the second molar ERR associated with the impacted third molar was 8.82%. ERR of the second molar was observed in 8 patients (7.84%) (Table 1). A statistical significance was not observed in the presence of ERR of the second molar in relation to the sex and age of patients (r = 0.818). The frequency of ERR of the second molar in the lower jaw is higher, and it is 8.2%, while in the upper jaw it is only 1% (r = 0.032) (Table 2).

There is a statistical significance of the second molar ERR presence in relation to the third molar position. Impacted third molars associated with the second molar ERR were most frequently in the horizontal (5.1%) and mesioangular (4.1%) position (p = 0.000). The second molar ERR frequency is also related to the depth of the third molar impaction. Impacted third molars associated with ERR of the second molar, were mostly in B (4.1%) and C (3.1%) class (r = 0.010).

There was a statistical significance of ERR severity related to the localization of resorption (r = 0.000). Mild resorption is more often present in the cervical and middle third of the root (I class), while the apical third is affected by the severe resorptions (III class). Resorption severity is significantly influenced by the third molar position (r = 0.000) and the depth (r = 0.008) of its impaction (Table 3).

The majority of ERR of the second molar belongs to the I class of resorption with 8.2%, while the III class of resorption was found in only 1% of cases. The II class of second molar ERR was not found at all (Table 4).

**Table 1**. Prevalence of the external resorption of the second molar roots in relation to sex and age groups

| Sex | Prevalence of external resorption | | | | Total | |
|---|---|---|---|---|---|---|
| | **Yes** | | **No** | | | |
| Male | 4 | 8,7% | 23 | 50% | 27 | 58,7% |
| Female | 4 | 8,7% | 15 | 32,6% | 19 | 41,3% |
| Total | **8** | **17,4%** | **38** | **82,6%** | **46** | **100%** |
| Age groups | | | | | | |
| 19-26 | 3 | 6,5% | 7 | 15,2% | 10 | 21,7% |
| 27-35 | 1 | 2,2% | 10 | 21,7% | 11 | 23,9% |
| >36 | 4 | 8,7% | 21 | 45,7% | 25 | 54,4% |
| Total | **8** | **17,4%** | **38** | **82,6%** | **46** | **100%** |

**Table 2.** Prevalence of the external resorption of the second molar roots in relation to the location of third molars

| **External resorption of the second molar** Note: analyzed by Fisher exact test | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **Yes** | | **No** | | **Total** | | **P value** |
| **Jaw** | | | | | | | |
| Upper | 1 | 1% | 46 | 46,9% | 47 | 48% | **0.032** |
| Lower | 8 | 8,2% | 43 | 43,8% | 51 | 52% | |
| **Third molar localization** | | | | | | | |
| Upper right third molar | 0 | 0 | 24 | 24,5% | 24 | 24,5% | **0.129** |
| Upper left third molar | 1 | 1% | 22 | 22,4% | 23 | 23,5% | |
| Lower left third molar | 4 | 4,1% | 21 | 21,4% | 25 | 25,5% | |
| Lower right third molar | 4 | 4,1% | 22 | 22,4% | 26 | 26,5% | |
| **The Winter classification** | | | | | | | |
| Horizontal | 5 | 5,1% | 5 | 5,1% | 10 | 10,2% | **0,000** |
| Vertical | 0 | 0 | 64 | 65,3 | 64 | 65,3% | |
| Mesioangular | 4 | 4,1% | 12 | 12,2% | 16 | 16,3% | |
| Distoangular | 0 | 0 | 8 | 8,2% | 8 | 8,2% | |
| **The Pel-Gregory classification** | | | | | | | |
| A | 2 | 2% | 61 | 62,2% | 63 | 63,3% | **0,010** |
| B | 4 | 4,1% | 19 | 19,4% | 23 | 23,5% | |
| C | 3 | 3,1% | 9 | 9,2% | 12 | 12,2% | |

**Table 3.** Prevalence of the external resorption of the second molar roots in relation to the position of the third molars

| Resorption classification Note: analyzed by Fisher exact test | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **No** | | **I** | | **III** | | **P value** |
| Jaw | | | | | | | |
| Upper | 46 | 46,9% | 1 | 1% | 0 | 0% | **0.043** |
| Lower | 43 | 43,9% | 7 | 7,1% | 1 | 1% | |
| Resorption localization | | | | | | | |
| Cervical third | 89 | 90,8% | 5 | 5,1% | 0 | 0% | **0.000** |
| Middle third | | | 3 | 3,1% | 0 | 0% | |
| Apical third | | | 0 | 0,0% | 1 | 1% | |
| The Winter classification | | | | | | | |
| Horizontal | 64 | 65,3% | 0 | 0,0% | 0 | 0% | **0.000** |
| Vertical | 5 | 5,1% | 4 | 4,1% | 1 | 1% | |
| Mesioangular | 12 | 12,2% | 4 | 4,1% | 0 | 0% | |
| Distoangular | 8 | 8,2% | 0 | 0% | 0 | 0% | |
| The Pel-Gregory classification | | | | | | | |
| A | 61 | 62,2% | 2 | 2% | 0 | 0% | **0.008** |
| B | 19 | 19,4% | 4 | 4,1% | 0 | 0% | |
| C | 9 | 9,2% | 2 | 2% | 1 | 1% | |

**Table 4.** Prevalence of localization and a class of the external root resorption of the second molar in relation to the localization and position of third molars

| | | |
|---|---|---|
| **Third molar localization** | | |
| Upper right third molar | 24 | 24,5% |
| Upper left third molar | 23 | 23,5% |
| Lower left third molar | 25 | 25,5% |
| Lower right third molar | 26 | 26,5% |
| **Resorption classification** | | |
| Without | 89 | 90,8% |
| I | 8 | 8,2% |
| III | 1 | 1% |
| **Resorption localization** | | |
| Without | 89 | 90,8% |
| Cervical third | 5 | 5,1% |
| Middle third | 3 | 3,1% |
| Apical third | 1 | 1% |
| **The Winter classification** | | |
| Vertical | 10 | 10,2% |
| Horizontal | 64 | 65,3% |
| Mesioangular | 16 | 16,3% |
| Distoangular | 8 | 8,2% |
| **The Pel-Gregory classification** | | |
| A | 63 | 63,3% |
| B | 23 | 23,5% |
| C | 12 | 12,2% |

## DISCUSSION

The second molar ERR associated with the impacted third molar is not a rare occurrence and it is often overlooked due to a lack of clinical symptoms. It is also considered to be one of the complications of impacted third molars (10). A cause of the third molar impaction is not exactly defined. One theory advocates an evolutionary reduction in the jaw size caused by lesser bone growth stimulation which is associated with a modern diet that has an inadequate chewing effect (16, 17).

In our study, ERR of the second molar associated with the impacted third molar was present in 7.84% of the cases, which is 8.82% of the examined teeth. Previous studies have shown that the prevalence of the second molar ERR associated with the impacted third molar ranges from 0.3-24.2%, by the application of the retro-alveolar radiography and orthopantomography (8). However, the prevalence of 20.2-54.9% ERR of the second molar was found by the application of CBCT (8). The Oenning et al study states that the prevalence of the ERR of the second molar is associated with the third molar impaction even higher (49.43%) by the application of CBCT versus standard radiographic methods (9). In the oral and maxillofacial surgery, the orthopantomographic scan is commonly used for the examination of the third molar position and its relation with the mandibular canal (18). Disadvantages of two-dimensional radiography are a poor resolution, image distortion, poor magnification and overlapping of anatomical structures. CBCT provides better diagnostic information in the sagittal, axial and coronary dimensions without overlapping of anatomical structures and pathological conditions, especially in the earlier stages of the formation (19, 20, 21). Alqerban and associates in their study suggested better detection of the resorption of the lateral incisor roots using the three-dimensional radiographic method (19).

Tassoke in his study showed a higher rate of ERR of the second molar in the lower jaw, which was also the case in our study (7). That study showed that there was 4 times greater risk for ERR in mandibular than in maxillary molars (7). This distribution is explained by the third molars eruption process in the lower jaw (7). There are many factors with an impact on that process like the limited skeletal and vertical condylar growth, a lack of space for the eruption due to an inadequate size of the crown, delayed eruption and the impossibility of adequate positioning of the third molar embryo (10,21).

By the application of the Winter classification, our study showed that the mesioangular and horizontal position of the impacted third molar has a greater risk for the second molar ERR. This result is consistent with results of previous studies (7,10, 12, 22 – 24). The least frequent third molar position was horizontal. Oenning and associates showed that there is no statistical significance in difference of the second molar external resorption in comparison with the mesioangular and horizontal third molar position. This result shows that a larger contact surface between horizontally impacted third molar crown and the second molar root does not represent a significant risk factor for external resorption (9). Our study also showed that there is no statistically significant difference in the second molar ERR formation, in relation to the mesioangular and horizontal third molar position.

Wang et al. showed the highest presence of the second molar ERR, based on the Pell-Gregory classification, in the class A (29.8% of cases), then the class C (24.5%), while the class B was the least represented (16.1% of cases) (12). Our study showed that the second molar ERR was classified mostly in the class B 4,1%, the class C 3% and the class A in 2% of cases.

Studies have shown that ERR is mostly localized in the cervical third of the second molar when localization of the third molar belongs to classes A and B (9). In our study, second molar ERR is most commonly localized in the cervical third (5.1%). The impacted third molars most often cause ERR in the apical third (25). In our study, only the apically localized resorption was in the class C.

Some studies showed a higher prevalence of the second molar ERR associated with the impacted third molar in male subjects due to the jaw or tooth size. Researchers explain this fact by the influence of sex hormones (26, 27). However, there are studies that show that the gender is not a risk factor for the second molar ERRs, which coincides with our results (7, 9). Wang et al. have shown in their research that the existence of the impacted third molar in the age group over 35 years is a risk factor for the second molar (12), while Oenning et al. have shown that this age limit is 24 years (9). This result of Wang et al. is possible due to the expected progression of the ERR second molar due to the prolonged retention of the impacted third molar (23).

There is no evidence to justify the prophylactic extraction of third molars unless there is an adequate clinical symptomatology. Although common complications of the impacted third molars are known, the scientific debate is raised whether the occurrence of the first clinical symptoms should be awaited for the preventive extraction. A lack of clinical confirmation of diagnosis found by CBCT is a limiting factor in our and other studies. Further research in this area is needed to obtain more precise results.

## CONCLUSION

The second molar ERR could be avoided by a preventive extraction of the mesioangular or horizontally placed third molars (16). An adequate decision for the extraction of impacted third molars must be made by careful assessment having in mind risks and benefits of this surgical intervention. Prior surgical intervention, an adequate care of the second molar, the restoration, the endodontic treatment or the root resection should be done if necessary (28 – 30).

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# THE DIVERSE AND HETEROGENEOUS CLINICAL FEATURES OF JUVENILE PSORIATIC ARTHRITIS

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# VARIJABILITET I HETEROGENOST KLINICKIH MANIFESTACIJA JUVENILNOG PSORIJATICNOG ARTRITISA

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## ABSTRACT

*Juvenile Psoriatic Arthritis (JPsA) is a chronic inflammatory disorder that accounts for 3-10% of all juvenile arthritis. The main objective is to identify the clinical features of psoriatic arthritis in children. This study was open-label, single-center, prospective, observational (1989-2020) cohort clinical study included 85 patients (3-17.0 y/o) who met Vancouver and I/E criteria. The features of skin and articular syndromes in children with JPsA were revealed. In most patients with PSA, the disease began at the age of 6.6 years. In childhood, arthritis is usually preceded by psoriasis. The most common clinical form of psoriasis is plaque psoriasis. At the onset of the disease, symmetric polyarthritis predominates. During the disease, transformation of the joint syndrome with a predominance of rheumatoid arthritis is noted. All observed patients showed pronounced osteoporosis, which is not characteristic of JPsA.*

***Keywords*:** *Vancouver diagnostic criteria, psoriasis, juvenile psoriatic arthritis, juvenile idiopathic arthritis.*

## SAŽETAK

*Juvenilni psorijatični artritis (JPsA) je hronični inflamatorni poremećaj koji čini 3-10% svih juvenilnih artritisa. Glavni cilj je da se identifikuju kliničke karakteristike psorijatičnog artritisa kod dece. Ova studija je bila otvorena, jednocentrična, prospektivna, opservaciona (1989-2020) kohortna klinička studija uključivala je 85 pacijenata (3-17,0 godina) koji su ispunjavali Vankuverske i I/E kriterijume. Otkrivene su karakteristike kožnih i zglobnih sindroma kod dece sa JPsA. Kod većine pacijenata sa PSA, bolest je počela u dobi od 6,6 godina. U detinjstvu, artritisu obično prethodi psorijaza. Najčešći klinički oblik psorijaze je psorijaza plaka. Na početku bolesti preovlađuje simetrični poliartritis. Tokom bolesti primećuje se transformacija zglobnog sindroma sa prevlašću reumatoidnog artritisa. Svi posmatrani bolesnici su pokazali izraženu osteoporozu, koja nije karakteristična za JPsA.*

***Ključne reči*:** *Vankuverski dijagnostički kriterijumi, psorijaza, juvenilni psorijatični artritis, juvenilni idiopatski artritis.*

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## INTRODUCTION

Psoriatic arthritis (PsA) is a chronic inflammatory joint disease that develops in about a third of patients with psoriasis (1). Juvenile Psoriatic Arthritis (JPsA) is presented in 4 - 9% of children with juvenile arthritis (2,3).

The criteria for the International League of Associations for Rheumatology (ILAR, 2001) and the Vancouver Criteria (1989) are used to diagnose JPsA (4, 5).

According to the ILAR criteria, Definite JPsA was defined as arthritis associated with psoriasis, or arthritis and at least 2 of the following: dactylitis, nail pitting or onycholysis, psoriasis in a first-degree relative. Exclusions criteria: arthritis in an HLA-B27 positive male beginning after the 6th birthday; ankylosing spondylitis, enthesitis related arthritis, sacroiliitis with inflammatory bowel disease, Reiter's syndrome, or acute anterior uveitis, or a history of one of these disorders in a first-degree relative; the presence of IgM rheumatoid factor on at least 2 occasions at least 3 months apart; the presence of systemic JIAin the patient (4). Psoriatic arthritis is a form of arthritis that affects some people with psoriasis. Psoriasis is a chronic skin and nail disease characterized by red, scaly rashes and thick, pitted fingernails. About one-third of children with psoriasis will have psoriatic arthritis. Psoriatic arthritis is a form of juvenile idiopathic arthritis (JIA) and is characterized by chronic joint inflammation and swelling, as well as an increased risk for asymptomatic eye inflammation. Psoriatic arthritis accounts for approximately 6 percent of all cases of juvenile arthritis. Although the cause of psoriatic arthritis is unknown, factors such as immunity and the environment may play a role. Genetics also appear to be involved: 40-80 percent of children with psoriatic arthritis have an affected first- or second- degree family member, such as a sibling, parent, grandparent or aunt/uncle.

Therefore, in pediatric rheumatological practice, the Vancouver criteria are more often used (5). According to Vancouver criteria Definite JPsA is defined as arthritis associated, but not necessarily coincident, with a typical psoriatic rash, or arthritis plus at least 3 of 4 minor criteria: dactylitis, nail pitting, psoriasis-like rash, or family history of psoriasis. Probable JPsA is defined as arthritis plus 2 of the minor criteria.

The clinical picture of JPsA is diverse and heterogeneous. There are no specific laboratory tests. Instrumental methods reflect only the presence and severity of the inflammatory process. JPsA is an interdisciplinary problem and requires the joint supervision of rheumatologists and dermatologists.

The main aim of this study was to identify the clinical features of psoriatic arthritis in children.

## METHODS

### Design of study

This study was open-label, single-center, prospective, observational (1989-2020) cohort clinical study included 85 patients (3-17.0 y/o) who met Vancouver and I/E criteria.

### Data collection presentation and follow-up

Patients′ charts were reviewed for the following clinical variables at presentation and at each follow-up visit: height, weight, joint involvement, presence or absence of symmetric arthritis, nail pits, dactylitis, presence of psoriatic rash or psoriasis-like rash, and uveitis. All me- dications, including corticosteroid joint injections, were recorded for each visit. Patient visits at presentation 1, 3, 6, 12, 36, and 60 months following the first presentation and every 5 years thereafter were used. Serologic variables studied were: a) Rheumatoid Factor (RF) as detected by latex agglutination; a positive test result was defined as titer ≥ 2 on at least two occasions (data were available on 88% of patients); b) Anti-nuclear antibody (ANA) as detected by immunofluorescent microscopy using the Hep2 cell line. A positive result was defined as a titer ≥ 1:40 on at least one occasion (data were available on 88% of patients); and c) HLA B-27 antigen (data were available on 44.5% of patients).

The following complications were recorded at each visit: presence of a joint contracture, and presence of leg-length discrepancy. The Childhood Health Assessment Questionnaire (CHAQ) (14) was available at last follow-up in 73.1% of the patients. For patients with uveitis the final visual acuity and ocular complications were obtained (15).

### Definitions

The diagnosis of psoriasis was made by a rheumatologist and/or dermatologist. Rashes thought likely (but not definitively) to represent psoriasis were considered psoriasis-like.

Polyarticular involvement was defined by the involvement of ≥ 5 joints cumulatively at any point over the course of study. Oligoarticular arthritis was defined as involvement of < 5 joints: a) persistent oligoarthritis- < 5 joints throughout the disease course; or b)extended- ≥ 5 were involved at any point after the initial 6 months. Enthesitis was defined as tenderness at a tendinous, ligamentous, capsular, or fascial insertion into bone. Dactylitis was defined as digital swelling extending be- yond the margins of the joints. Patients with dactylitis were not considered to have involvement of the corres-ponding joint(s) with arthritis unless it was specifically documented.

Symmetric arthritis was defined as being present if the number of affected joint pairs divided by the total number of joints involved was ≥50%. The fol- lowing 11 joint pairs were used: shoulders, elbows, wrists, any metacarpophalangeal (MCP), any pro- ximal interphalangeal (PIP) of the

hand, hips, knees, ankles, any metatarsophlangeal (MTP), any PIP) of the foot, and the temporomandibular (TMJ).

**Statistic methods**

All descriptive data were expressed as the mean ± standard deviation (SD). Comparisons between groups were performed using Chi-Square Tests with Bonferroni correc- tions. All data are presented in form of the Tables and Figures.

## RESULTS

In this study we observed 85 patients with JPsA aged from 3 to 17 years. Fifty-nine (70%) patients had definite JPsA and 24 (20%) had probable JPsA, according to the Vancouver criteria. The clinical and demographic characteristics are presented in Table 1.

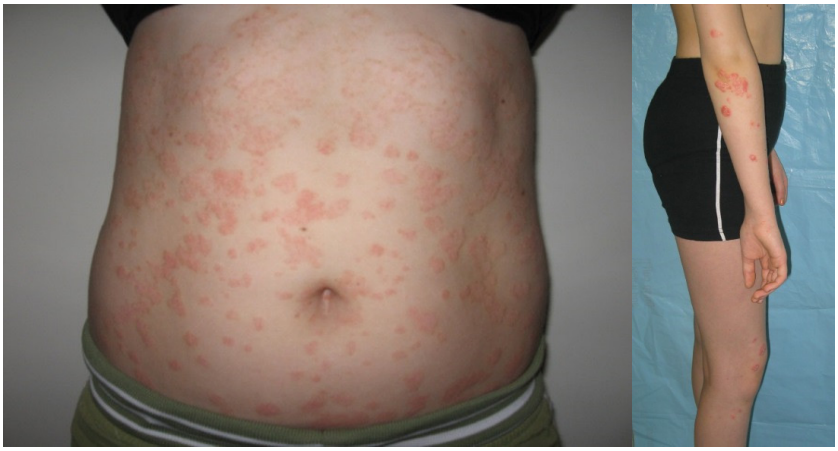
**Table 1**. The clinical and demographic characteristics of patients with JPsA (n=85)

| **Demographic indicators** | | **JPsA** | |
|---|---|---|---|
| | | **Definite** | **Probable** |
| Number of patients (n) | | 60 | 25 |
| Girl/Boy Ratio | | 1,7:1 | 2:1 |
| Average age, years | | 6,6±4 | 6,3±2,7 |
| Duration of the disease, years | | 3 ±2 | |
| **Family history of psoriasis n, (%)** | First-degree relative with psoriasis | 22 (26%) | |
| | Second-degree relative with psoriasis | 34 (41%) | |
| **Potential trigger n, (%)** | Infection | 22 (27%) | |
| | Trauma | 12 (14%) | |
| | Vaccination | 7 (8%) | |
| | Insolation | 5 (6%) | |
| | Stress | 3 (4%) | |
| | Not identified | 34 (41%) | |

In 18 (30%) children with definite JPsA, skin lesions presented as the first sign of the disease, joint damage in these patients developed after 3.5 ± 2 years. In 29 (49%) patients' articular syndrome was observed on the onset with subsequent skin manifestations after 5.3 ± 3 years. Fourteen (23%) children with definite JPsA had simultaneous debut of skin and articular syndromes.

Among 60 patients with definite JPsA, vulgar psoriasis was observed in 46 (76%) patients, guttate psoriasis in 10 (15%), isolated nail psoriasis in 3 (5%), and palmoplantar psoriasis in 2 (4%). Fifteen (28%) patients had a combination of cutaneous psoriasis with damage to the nail plates (Figure 1).

**Figure 1.** Vulgar psoriasis in patient with JpsA



Articular syndrome in the onset of the disease was represented by oligoarticular arthritis in 58 (70%) patients, in 16 (19%) children - symmetric rheumatoid-like arthritis, and in 11 (13%) - spondylitis. The most commonly involved joints at both presentation and during the course of the disease were the knee (41%), ankle (31%), and small joints of the hands (29%). During course of the disease, the articular syndrome transformed with symmetric rheumatoid-like arthritis prevalence.

All joint groups in PsA may be involved in the pathological process. However, knee joints, ankles, and interphalangeal joints are more often affected (41.0%, 31.3%, 28.9%, respectively) (Table 2, Figure 4).

**Table 2.** Joint disease incidence in PsA children (n=85)

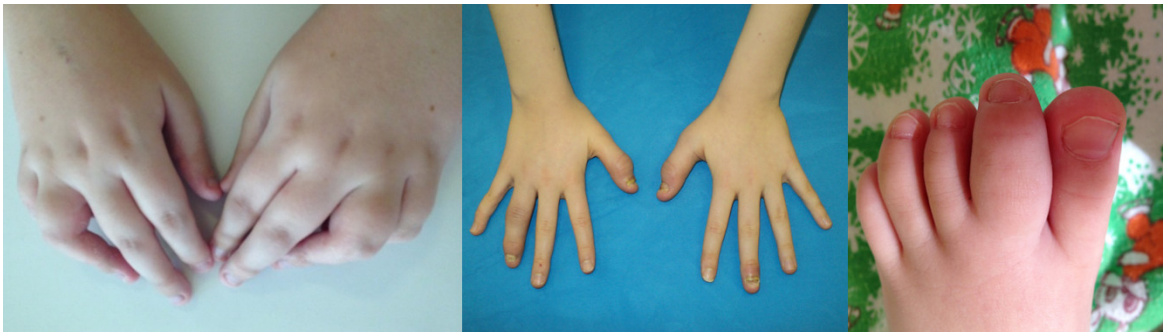
| Affected joints | Absolute number, n | % |
|---|---|---|
| Axial skeleton (cervical, thoracic, lumbar spine sections) | 6 | 10,8 |
| Temporomandibular joint | 1 | 1,2 |
| Shoulder joints | 6 | 7,2 |
| Elbow joints | 9 | 10,8 |
| Wrist joints | 10 | 12,1 |
| Small joints of the hands | 24 | 28,9 |
| Hip joints | 8 | 9,6 |
| Knee joints | 34 | 41,0 |
| Ankle joints | 26 | 31,3 |
| Small joints of the feet | 15 | 18,1 |
| Enthesopathies | 4 | 4,8 |

**Figure 2.** Joint damage in JpsA

Mutilans arthritis

Arthritis of the distal interphalangeal joints. Psoriatic damage to the nail plates

Axial arthritis



According to our data, the clinical picture of PsA turned out to be variable. In 40 (47%) children, the disease was characterized by high laboratory and clinical activity. Exacerbations of articular and skin syndromes were noted up to 5-6 times a year. In 45 (53%) patients, on the contrary, the process was easy, there was a positive effect from the ongoing anti-rheumatic therapy.

According to the results of the X-ray examination, in the active period of the disease, 40 (45%) patients had the I radiological stage according to Steinbroker, in 44 (53%) the III-IV radiological stages.

It should be noted that in all patients, periarticular osteoporosis was expressed, which is not characteristic of PsA.

## DISCUSSION

Despite the current experience and knowledge in the field of PsA, the etiology and pathogenesis are still largely unclear. Environmental factors, including infection, trauma, stress, only trigger factors in the development of PsA in genetically predisposed people.

So, according to the results of our study in most cases (41%), the potential trigger could not be established. A hereditary predisposition to the development of psoriasis and PsA are noted: it is known that more than 40% of patients have a family history of psoriasis in relatives of the 1st degree of kinship. According to our data, a family history of psoriasis among relatives of the 1st degree of kinship was observed in 26% of children, 2nd degree of kinship - in 41% of children. Girls predominate in the sexual distribution, which is consistent with literature (6, 7, 8). However, two peaks of morbidity at an early age and adolescence described in the literature are not traced by us. In childhood, according to a few studies, in 50% of cases, arthritis precedes the appearance of psoriasis (9, 10). So, according to our data, in 48.0% of children, articular syndrome preceded psoriasis. In children with JPsA, asymmetric oligoarthritis is more often observed at the onset of the disease (9,10,11,12). Among the children we observed in the onset of the disease, asymmetric oligoarthritis was recorded in 69% of children. During the course of the disease, a transformation of the articular syndrome was observed with rheumatoid arthritis prevailing (46.6%). The most common clinical form of psoriasis was plaque psoriasis (71.2%), which is consistent with published data. (13,14,15). Much less often than in the described literature, an isolated lesion of the nail plates was found (5% versus 20-50%) (15). In 40-60% of patients, biomarkers of inflammation (ESR and CRP) remain normal (16). In our study, in 20% of children, the disease proceeded without activity. The X-ray picture in PsA is characterized by features: narrowing of the joint gap, bone remodulation (resorption of terminal phalanges, large eccentric erosion, osteolysis), bone proliferation (marginal bone growths, periostitis, enthesophytes, bone ankylosis), asymmetric bilateral or unilateral spondylitis, paravertebral ossification and marginal syndesmophytes (17). According to our data, 53% of patients had the III-IV radiological stages, 47%) patients had the I radiological stage according to Steinbroker. Periarticular osteoporosis has been described in all children with JPsA, which is not characteristic of JPsA (18).

## CONCLUSION

The clinical picture of JPsA is variable. Most patients with JPsA do not have a chronological relationship between skin and joint damage. The most common form of psoriasis is plaque psoriasis. At the onset of the disease, asymmetric oligoarthritis prevails, followed by transformation with a predominance of rheumatoid-like arthritis. Thus, children with JPsA require the joint supervision of a rheumatologist and a dermatologist.

## CONFLICT OF INTEREST

None.


## FUNDING

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# PHYTOCHEMICAL AND PHARMACOLOGICAL PROPERTIES OF ALLIUM URSINUM

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# FITOHEMIJSKE I FARMAKOLOŠKE KARAKTERISTIKE *ALLIUM URSINUM* (SREMUŠA)

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## ABSTRACT

*Allium ursinum L. is a perennial herbaceous plant belonging to the Alliaceae family. Several classes of bioactive compounds have been isolated from A. ursinum so far, among them sulfur and phenolic compounds as quantitatively the most distributed constituents, responsible for pharmaceutical effects of the plant. Small amounts of steroidal glucosides, lecitins, fatty acids and several amino acids, as well as the essential oil are also present in A. ursinum. This plant species is characterized by a long history of use in traditional medicine in the prevention and treatment of cardiovascular disease, as digestive stimulant, antimicrobial agent, as a remedy in respiratory problems, insomnia and fainting. Despite its widespread use for medicinal purposes since the ancient time, studies referring to its pharmacological activity are still lacking. In this review, we summarized the current knowledge related to the phytochemical and pharmacological properties of Allium ursinum. This study may be a starting point for future researches in this field, which would fully clarify therapeutic potential of A. ursinum and make it a possible candidate for medicinal product.*

**Keywords**: *Allium ursinum, wild garlic, ramson, biological activity, chemical composition*

## SAŽETAK

*Sremuš je višegodišnja zeljasta biljka koja pripada porodici Alliaceae. Do sada je iz sremuša izolovano nekoliko klasa biološki aktivnih jedinjenja, među kojima su fenolna i jedinjenja sumporna kvantitativno najzastupljenija i odgovorna za farmakološke efekte ove biljne vrste. Male količine steroidnih glikozida, lecitina, masnih kiselina i aminokiselina, kao i etarskog ulja su takođe prisutna u sremušu. Ova biljna vrsta se karakteriše dugom istorijom upotrebe u tradicionalnoj medicini u prevenciji i lečenju kardiovaskularnih bolesti, kao lek kod respiratornih tegoba, nesanice i nesvestice. I pored njene široke primene u medicinske svrhe od davnih vremena, I dalje nedostaju istraživanja koja se odnose na njenu farmakološku aktivnost. U ovom preglednom radu sumirana su trenutna saznanja vezana za fitohemijske i farmakološke karakteristike sremuša. Ova studija može biti osnova za dalja istraživanja u ovoj oblasti, koja bi u potpunosti razjasnila terapijski potencijal sremuša i učinila ga mogućim kandidatom za medicinski proizvod.*

**Ključne reči**: *Sremuš, divlji luk, medveđi luk, biološka aktivnost, hemijski sastav*

## INTRODUCTION

*Allium ursinum L.* (synonyms: wild garlic, ramson or bear garlic) is a perennial plant species which grows in fens and river woods of Europe and Asia. However it isn't distributed in different areas in Russia, but it can occur in Mediterranean region (1, 2). The Latin species name "ursinum" has been derived from "ursus" (bear) since there is a belief that the first thing bears eat after awakening from winter hibernation to remove toxins is exactly this herb (3). *A. ursinum L.* has been used for centuries in traditional medicine both as prophylactic and therapeutic agents. In the past two decades consummation of this plant species as a dietary supplement and food has gained attention of modern medicine due to its various health benefits (3, 4). Published papers referring to its chemical constituents and protective effects on health place his positive activity even above *Allium sativum* (garlic), the most famous plant from *Alliaceae* family (5). Numerous biologically active compounds are presented in this herb such as: sulphur

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compounds, phenolics, steroidal glycosides etc., however sulphur compounds mostly contribute to its pharmacological activity (3, 6, 7). Despite the history of traditional applications of *Allium* species as both condiments and phytopharmaceuticals, researches based on its composition and pharmacological activity are still lacking. The aim of our study was to summarize the current knowledge referring to the chemical constituents and biological activity of *A. ursinum* and to highlight its therapeutic potential that has been recognized in traditional medicine from ancient times.

**Description**

There are two subspecies of *A. ursinum* which *differs* in pedicel morphology: *A. ursinum* ssp. *ursinum* and *A. ursinum* ssp. *Ucrainicum.* First one is distributed in western and middle Europe, while the second one is distributed in eastern and southern Europe (8-10). *Both of them grow* up to the height of 50 cm in deciduous woodlands with nutrient-rich and moist soils. It is characterized by elongated narrow bulbs (1.5-6 cm in size), dark green leaves with a distinct garlic-like scent (up to 50 cm long) and white flowers (11, 12). . The whole herb is edible and curative. Active growth of *A. Ursinum begins in late February and early March, when there is* enough of light. Afterwards the leaves are collected on April and May, while the blooming period lasts from April until the first half of the May (3, 13). Restricting factors for their distribution are the presence of aluminum in high concentration in soil (14).

**Chemical constituents**

Previosuly conducted studies revealed the presence of sulfur and phenolic compounds, steroidal glycosides etc in *A. ursinum* (3, 6, 7).

**Sulfur compounds**

The most important chemical constituents present in *A. ursinum* that has been considered to mostly contribute to therapeutic benefits of the plant are sulfur compounds (6). Characteristic smell and taste of this plant derives from alliin and γ-glutamylcysteines, representatives of a special class of pharmacologically active organo-sulfuric compounds *S*-alk(en)yl-l-cysteine-sulfoxides. Plant disruption leads to a hydrolysis of those non-volatile secondary metabolites and formation of volatile alk(en)yl-thiosulfinates, such as allicin and *lipid-soluble* allyl *sulfur compounds such* as *diallyl disulfide* (DADS) and *diallyl trisulfide* (DATS) (15, 16). Allicin and methyl-allyl- or dimethyl thiosulfinates are the major thiosulfinates that can be detected after hydrolysis of cysteine sulfoxides. Nevertheless thiosulfinates are unstable and decomposes rapidly to (poly) sulfides, dithiins, ajoenes etc (11, 17).

The presence of methiin ((+)-*S*-methyl-l-cysteine-sulfoxide), alliin ((+)-*S*-2-propenyl-l-cysteine-sulfoxide), isoallin ((+)-*S*-(1-propenyl)-l-cysteine-sulfoxide), propiin ((+)-*S*-propyl-l-cysteine-sulfoxide) and ethiin (*S*-ethyl-cysteine-sulfoxide) has been proved in wild garlic. Since methiin and alliin has been found in the highest quantities *A. ursinum* is considered to belong to a methiin/alliin-type *Allium* species (3, 18).

Time of harvest affects the amount of cysteine-sulfoxides in this herb, thus suggesting that the highest concentration would be achieved if it was collected in March and April (12, 19). Sulfur-containing compounds, with the highest amount of allyl polysulfides, have also been identified in the essential oil of *A. ursinum* (12, 20-22).

**Phenolic compounds**

The presence of phenolic compounds, predominantly kaempferol derivatives, has also been proved in *A. ursinum.* Phytochemical investigation of ethanol extract of wild garlic leaves has led to the isolation of : kaempferol 3-O-beta-neohesperidoside-7-O-[2-O-(trans-p-coumaroyl)]-beta-D-glucopyranoside, kaempferol 3-O-beta-neohesperidoside-7-O-[2-O-(trans-feruloyl)]-beta-D glucopyranoside, kaempferol 3-O-beta-neohesperidoside-7-O-[2-O-(trans-p-coumaroyl)-3-O-b eta-D- glucopyranosyl]-beta-D-glucopyranoside, kaempferol 3-O-beta-glucopyranoside and kaempferol 3-O-beta-neohesperidoside (23). Furthermore, extract prepared from dry leaves of *A. ursinum* (fresh leaves were freeze dried) contained 3-*O*-β-d-glucopyranoside, 3-*O*-β-d-glucopyranosyl-7-*O*-β-d-glucopyranoside, 3-*O*-α-l-rhamnopyranosyl-(1 → 2)-[3-acetyl]-β-d-glucopyranoside and 3-*O*-α-l-rhamnopyranosyl-(1 → 2)-[6-acetyl]-β-d-glucopyranoside in addition to several of the previously mentioned compounds (24). Another study has reported that fresh flowers of wild garlic contain the following constituents: thymidine, adenosine, astragalin (kaempferol-3-O-beta-D-glucopyranoside, kaempferol-3-O-beta-D-glucopyranosyl-7-O-beta-D-glucopyranoside, kaempferol-3-O-beta-D-neohesperoside and kaempferol-3-O-beta-D-neohesperoside-7-O-beta-D-glucopyranoside (21).

It has been confirmed that the yield of *phenolic* compounds depends on extraction method, extraction solvent and *part of the plant used* for *extraction.* A previously conducted research indicate that total polyphenol content in the leaf extract was higher when the extract was prepared by maceration with 70 % ethanol in comparison with the ultrasound-assisted extraction (25). Qualitative differences in the phenolic fractions between acidified methanol extracts of green and yellow leaves, stalks, and seeds of wild garlic exist, suggesting that the content of the kaempferol derivatives and compounds acylated with p-cpumaric acid were the highest in yellow leaves followed by green leaves. Generally viewed, seeds contained the minimal amount of phenolic compounds, however more non-acetylated in comparison to acetylated flavonoid glycosides with p-coumaric acid compounds were found in stalks and seeds compared to leaves (26). Additionally other group of authors has revealed that total free phenolics content in bulbs

was lower than in leaves, however the amount of bound phenoics were similar (27). The highest content of gallic acid was found in 96% methanol extract of *A. ursinum*, followed by 80% methanol extract, while the lowest was noticed in 96 % ethanol extract (28). Furthermore the ecotypes of *A. ursinum* differ in chemical composition (29). The possible discrepancy in the amount of phenolics found by different authors may be the consequence of different methods used for quantification.

**Steroidal glycosides**

Phytochemical researches have reported the common presence of steroidal glycosides in *A. ursinum*. Diosgenin 3-*O*-α-l-rhamnopyranosyl-(1→4)-α-l-rhamnopyranosyl-(1→4)-[α-l-rhamnopyranosyl-(1→2)]-β-d-glucopyranoside and (25*R*)-spirost-5,25(27)-dien-3β-ol 3-*O*-α-l-rhamnopyranosyl-(1→4)-α-l-rhamnopyranosyl-(1→4)-[α-l-rhamnopyranosyl-(1→2)]-β-d-glucopyranoside has been isolated from the bulbs of wild garlic. Furthermore, a pregnane glycoside such as 3-hydroxy-pregna-5,16-dien-20-on 3-*O*-α-l-rhamnopyranosyl-(1→4)-α-l-rhamnopyranosyl-(1→4)-[α-l-rhamnopyranosyl-(1→2)]-β-d-glucopyranoside were found in the underground parts of wild garlic (30).

**Other compounds**

The major *predominant* biologically active substances in *A. ursinum* responsible for the effects of *A. ursinum* on maintaining good health are undoubtedly sulfur and phenolic compounds. However there are other chemical constituents presented in this herb: lectins, polysaccharides, fatty acids (palmitic, linoleic, oleic, palmitoleic, stearic, α-linolenic, and myristic acid), many amino acids (asparagine, glutamine, aspartic acid, glutamic acid, arginine, alanine, glycine, threonine), pigments etc (3, 31, 32).

***A. ursinum usage***

There is an evidence that *A. ursinum* has been extensively used worldwide in the treatment of many disease and conditions even since the Mesolithic period (33). All parts of the plant are edible either raw or cooked and becoming increasingly popular in local cuisines of Europe. Leaves may be used as a salad, spice, boiled as a vegetable. Therapeutic potential of *A. ursinum* is attributed to leaves, herb and bulbs, which are at the same time the most commonly used in traditional medicine (3, 26).

**Pharmacological effects of *A. ursinum***

The main application of wild garlic is in the prevention and treatment of cardiovascular diseases and as antimicrobial agent.

***Effects of A. ursinum on cardiovascular diseases***

*A. ursinum* and its preparations play one of the most important role in prevention and treatment of cardiovascular diseases. The relevant literature showed that consumption of ramson significantly decreased blood pressure, serum cholesterol and triglyceride. Furthermore, this wild plant inhibits platelet aggregation due to the presence of flavonoids and prevents progression of atherosclerosis. Experimental as well as clinical studies demonstrated these favorable cardiovascular effects when various preparation of *A. ursinum* was used (2, 3, 27).

Groundbreaking in vitro investigation conducted in 1992 brought novel information about potential effect of wild garlic aqueous extract on blood pressure. This group of authors showed that leaf water extract administered in concentration of 0.3 mg/kg inhibited angiotensin 1-converting enzyme (ACE) almost two times higher than garlic leaf extract. Assumption for ACE inhibition may be due to gamma-glutamyl peptides which are presented in wild garlic two times more than in cultivated garlic (34). One year later Rietz and co-workers examined plasma ACE activity and size of ischemic zone and ischemia/reperfusion induced arrhythmias. In wild garlic group ACE activity was significantly reduced in comparison to control group. In this study rats were on standard diet with 2 % of pulverized A. ursinum leaves for 8 weeks (35). Hypertensive effect of *A. ursinum* was noticed in experiments conducted on Spontaneously Hypertensive Rats. In this investigation rats were divided into following groups: control group fed with standard diet, group fed with diet containing 1% w/w wild garlic and group fed with diet containing 1% w/w garlic for 45 days when SBP were measured. Reduction of final mean systolic blood pressure (SBP) in wild garlic group was 173 ± 0.7 mmHg in comparison to control group 189 ± 1.2 mmHg. Despite the fact that wild garlic was used in lower concentration 0,1% w/w, SBP was significantly lower compared to rats which consumed garlic in the same concentration (5). It has been suggested that hypotensive effect of wild garlic may be due to activation of nitric oxide system and decreased activation of RAS (36).

It has been reported that *A. ursinum* essential oil increased fluidity of deep layers of the artificial liposome membrane. Also they claimed that ramson essential oil may be involved in the regulation of membrane functions in hypertension, as well as an antioxidant (37). In addition one group of researchers conducted investigation on monocrotaline (MCT) induced pulmonary arterial hypertension rat model by comparing 2% wild garlic liophylisate-supplementation to 25 mg/kg sildenafil orally administred for 8 weeks. Findings from this study suggest beneficial effect of wild garlic demonstrated by echocardiographic and isolated heart functions, or histological analyses (38).

As mentioned above, allicin (allyl 2-propenethiosulfinate or diallyl thiosulfinate) belongs to tiosulfinates, main compounds of *A. ursinum* produced by influence of allinase on allin. Some researchers described hypotensive ef-

fect of allicin in Spontaneously Hypertensive Rats as well as in CKD chronic kidney disease rat model. Allicin has been proven to abolish oxidative stress by preventing the generation of free radicals and exert cardioprotective effects likely through downregulation of angiotensin II receptor type 1 (AT1R) and Keap1 expression (39).

***Antimicrobial activity***

Numerous number of studies support the fact that *A. ursinum* may be used both internally and externally as an antimicrobial agent. Extracts prepared from fresh flowers and leaves exert antifungal and antibacterial activity, predominantly oriented on Gram (+) and less on Gram (-) bacteria (40, 41). Antimicrobial potential of this plant is attributed to sulfide compounds, especially allicin with proven antifungal effect (42).

Flowers and leaves of *A. ursinum* possess different antifungal potential, thus suggesting that flower extract is more *efficient* antifungal agent (41). Additionally, antibacterial, antifungal and antiparasitic activity of bulb juice and water extract from bulbs of *A. ursinum has also been revealed (3,* 43, 44).

Antibacterial and antifungal activity of the plant extract depends on the extraction solvent, thus suggesting that higher effect is expected from methanol compared to water extract. Methanol extract has been shown to exhibits antimicrobial activity against both Gram (+) and Gram (-) bacteria including: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Proteus mirabilis*, *Salmonella enteritidis* and fungi such as : *Cladosporium* sp., *Aspergillus niger*, *Rhizopus nigricans*, *Geotrichum candidum*, *Penicillium expansum*, *Candida lipolytica*, *Mycoderma*, *Saccharomycopsis fibuligera.* On the other hand water extract was efficient only against B. Subtilis. (3).

***Other pharmacological effects***

*A. ursinum* is efficient in treatment of respiratory tract problems (cold, fever, bronchitis), as digestive stimulant, vulnerary antidiarrheal, antiphlogistic, antimicrobial agent (3, 45). Furthermore, its effect in removal of toxic substances from the organism has been also recognized since the ancient time (24) Researches suggested that *A. ursinum* may inhibit the activity of 5-lipoxygenase and cyclooxygenase (46). The chloroform extract from flower stems showed the most promising cytotoxic effect against murine cancer cell lines melanoma B16 and sarcoma XC (Trypan Blue Exclusion Test of Cell Viability) (47).

This plant possess antioxidant activity due to the presence of phenolic compounds and high antioxidant enzymes activities, including catalase, glutathione peroxidase and superoxide dismutase. In that sense, consummation of *A. ursinum* protects cell proteins and membranes against oxidation and consequential damage (2, 32, 45, 48).

## CONCLUSION

The results of the mentioned studies clearly demonstrate that wild garlic exert myriad beneficial effects mainly attributed to sulfur compounds, thus absolutely justifying its widespread traditional use. However further studies in this field are necessary in order to fully clarify its therapeutic potential and make it become a source for pharmaceutical exploitation.


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## CONFLICT OF INTEREST

The authors declare there is no actual conflict of interest.

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# PROSTHODONTIC REHABILITATION OF PATIENTS WITH DOUBLE CROWN AND LOCATOR ATTACHMENT - RETAINED OVERDENTURES SUPPORTED BY A COMBINATION OF NATURAL TOOTH AND STRATEGIC IMPLANTS: CASE SERIES

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# PROTETSKA REHABILITACIJA PACIJENATA SUPRADENTALNIM PROTEZAMA PODUPRTIH KOMBINACIJOM PRIRODNIH ZUBA I DENTALNIH IMPLANATA, SA DVOSTRUKIM KRUNAMA I ATEČMENIMA TIPA LOKATORA: SERIJA SLUČAJEVA

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## ABSTRACT

*Prosthetic rehabilitation of edentulous patients and patients with one or two own teeth can be established by different treatment modalities. The most commonly used in the treatment of these patients is conventional complete denture or removable partial denture. However, due to increasing problems with this type of therapy, such as insufficient retention, stability, comfort and pain during mastication, it is suggested an overdenture supported by two natural teeth or implants. We will present series of clinical reports. In two clinical cases patients came to the dental office because of the impossibility of wearing lower partial denture, and in one case patient had problems with the upper partial denture. After clinical examination and radigraphic analysis, in all patients, dental implants were implanted. In first case there were implanted two dental implants in the region 41 and 43, in second case it was region 33, and in third case implanatiton is performed in the region of 14, 11, 21. Prosthetic rehabilitation was done after 3-months bone oseointegration period. The treatment consisted in the production of double crowns and overdentures that are retained with locator attachment. This design of the denture significantly improves the quality of patient's life (the dentures are stable, chewing is improved, the feeling of thermal sensations of food and drink is present, the feeling of taste is complete, and the psychological patient becomes safer).*

***Keywords**: overdenture, double crown, locator, dental implant.*

## SAŽETAK

*Protetska rehabilitacija bezubih pacijenata i pacijenata sa jednim ili dva svoja zuba može se izvršiti različitim tarpijskim modalitetima. U terapiji ovih pacijenata najčešće se koristi konvencionalna totalna proteza ili parcijalna proteza. Međutim, zbog sve većih problema sa ovom vrstom terapije, kao što su nedovoljna retencija, stabilnost, udobnost i bol tokom žvakanja, predlaže se izrada supradentalne proteze koja je poduprta sa dva prirodna zuba ili implantata. Ovde je prikazana serija kliničkih slučajeva, u dva klinička slučaja pacijenti su došli u stomatološku ordinaciju zbog nemogućnosti nošenja donje parcijalne proteze, dok je u jednom slučaju pacijent imao problema sa gornjom parcijalnom protezom. Nakon kliničkog pregleda i radiografske analize, kod svih pacijenata ugrađeni su dentalni implantati. U prvom slučaju ugrađena su dva implantata u području 41 i 43, u drugom slučaju u području 33, a u trećem su ugrađeni u regionu 14, 11, 21. U svim slučajevima nakon perioda oseointegracije od 3 meseca izvršena je protetska sanacija. Tretman se sastojao od izrade dvostrukih krunica i supradentalnih proteza koje su retinirane lokator atečmenima. Ovaj dizajn proteze značajno poboljšava kvalitet života pacijenta (proteze su stabilne, žvakanje je poboljšano, osećaj termičkih senzacija hrane i pića je prisutan, osećaj ukusa je potpun, a psihološki pacijent postaje sigurniji).*

***Ključne reči:** supradentalna proteza, dvostruka kruna, lokator, implantat.*

## ABBREVIATIONS

**CBCT** - Cone beam computed tomography
**RPD** - Removable partial denture

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## INTRODUCTION

Prosthetic rehabilitation of an edentulous mandible can be established by using different treatment modalities. Conventional complete dentures are a common prosthodontic treatment used for the replacement of all missing teeth. However, patients wearing complete denture often suffer from a variety of problems with their dentures, especially in the lower jaw, such as insufficient retention, stability, comfort and pain during mastication. With time, the resulting difficulties can compromise patient's oral functions (choice and enjoyment of food, chewing efficancy and nutritional intake), esthetics and may result in a range of emotional reactions in edentulous people (1,2). Also, complete denture does not stimulate alveolar bone adequately as natural teeth does. The lack of periodontal mechanoreceptors can lead to overloading of denture bearing areas during masticatory function resulting in higher resorption rate (3,4). For these reasons, conventional complete denture have been modified to gain additional support and stability from a few retained natural teeth or implants. The use of an overdenture supported by two natural teeth or implants shows some advantages over the complete denture treatment such as decreased resorption of the alveolar ridge (5,6), improved retention and stability of the dentures, grater satisfaction and improved patients' quality of life (7). Such caracteristics associated with the high success rate in implant treatment (8,9), make the overdenture retained by two implants the standard treatment for the edentulous mandible.

Many types of attachments can be used for connecting overdenture to natural teeth and implants (10,11) and their correct selection is very important for successfull prosthetic treatment. Telescopic crowns as retainers between natural teeth and overdentures have been used successfully in clinical practice (10). This type of retainer provides good retention and stability of overdenture, the transmission of masticatory load along the long axis of the abutment teeth, maintainance sensory feedback and protection from dislodging movement. Apart from being used as retainers for connecting natural teeth and overdenture, telescopic crowns have been used to retain implant-supported overdentures with long-term denture and implants survival rates (11,12,13). Also, mandibular implant-supported overdentures may be successfully retained with ball, bar, locator and magnetic attachments (14).

In unfavorable prosthetic baseline situations with reduced number of abutment teeth, the use of implants in strategic positions on the opposite side of the dental arch is nedeed to equalize the balance and create suitable support and retention. The combined use of implant and natural teeth to support an overdenture presents a certain biomechanical challenge due to the dissimilar mobility between the abutments and different mechanism of absorption and dissipation of forces on supporting tissues. This treatment option has been disputed in dental literature (15,16), but it offers an extensive and almost unlimited spectrum of new treatment possibilities (17,18). Therefore, this clinical report describes the prosthetic rehabilitation of patients with double crown and locator attachments-retained overdentures supported by a combination of natural tooth and strategic implants.

## CASE REPORT 1

The 67-year-old patient came to the dental office for impossibility of wearing the lower removable partial denture (RPD). Based on patient`s history and clinical examination, inadequate stabilization and retention of lower RPD was revealed. The analysis of the anamnestic questionnaire found that the patient does not have general chronic diseases and does not use any type of therapy. Implant planning and implantation of dental implant was performed on the basis of Cone beam computed tomography (CBCT) analysis and evaluation. In the dental status in the lower jaw on the left side there was an endodontically treated caninus, whose periodontal status was good. In the upper jaw there was combined prosthetic restoration with attechments. After implantation of dental implant in the region 41 and 43 (Implant direct legacy 3, USA), and the 3-month bone oseointegration period, the prosthetic rehabilitation of the patient was done. The tooth is reinforced with Fiber-reinforced composite posts (FRC Postec Plus, size 1, Ivoclar, Vivadent, Liechtenstein) and cemented with composite cement (Multilink Automix, Ivoclar, Vivadent, Liechtenstein).

Before the start of prosthetic rehabilitation, both implants were opened and gingival binders were implanted. In the first phase of prosthetic rehabilitation, preparation of the tooth 33 was done, and the impression was taken by double method using addition elastomeric material (Elite P & P Putty Soft, Elite HD + Regular Body Normal Set, Zhermack, Italy) with the previous application of retraction thread (Ultrapak No 0, Ultradent, USA). In the same visit, the depth of the sulcus for future locators was measured (the implant platform for the locator was 3.5 mm and the height of both locators was 2 mm). After the telescope double crown system was completed (milling of the inner telescope was 6°), an inner telescope crown was examined and its attachment to the prepared tooth was checked, after which an outer telescope crown was made. Then the relation of the outer and inner telescopes to the prepared tooth was checked. Prior to taking a functional impression, locators implant attachment of mentioned dimensions (Zest Dental Solutions, USA), which are tightened on the 30Ncm with torque wrench, were set up using the locator square driver 15mm wrench. Also, female parts of the locator with working tires (black color) were set up, after which the anatomical impression was taken. On an anatomical model, an individual tray with interspace was made, according to all principles of extension as for full denture. After the verification of the telescope, a functional impression of lower jaw was taken using elastomeric impression material (the functional impression was taken by all principles as for full denture), thermoplastic mass (Impression Compound, Harvard, Germany) was used for shaping the edges of the individual tray and the elastomeric impression material in the individual tray (Xantopren M mucosa, Heraeus Kulzer

GmbH, Germany), using an adhesive for individual tray (Universal Adhesive, Heraeus, Germany). The reason for taking definitive impression over the female parts of the locator is that the technician makes perforations in the framework and later the interspace for cold-polymerizing acrylate for the direct attachment of the denture to the female part of the locator.

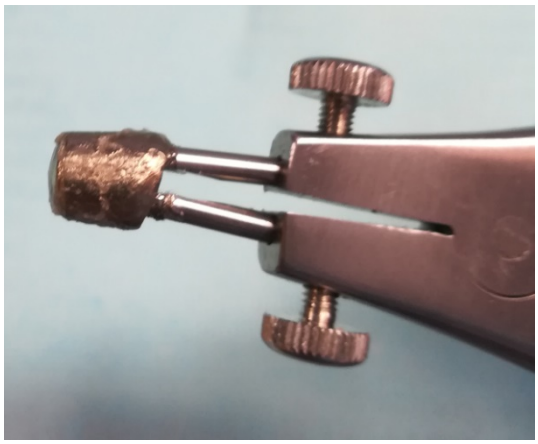
Outpouring a functional impression it was obtained a working model for making of a RPD framework, and later a model for completing this prosthetic restoration. After RPD framework was completed, in dental office cheking of framework, determination of jaw relationship and the colour of the teeth was done. In next phase cheking of teeth setting, verification of jaw relationship, as well as shape and position of the teeth was done, after which restoration was sent to the laboratory for the definitive ending.

The specificity of the ending of restoration in clinical work is that the female part of the locator is first connected with a cold-polymerizing acrylate for denture (prior it is necessary to prepare the locator system, a white ring is placed on it, that prevents onflow of liquid acrylate along the abutment of locator, and female part of the locator is placed over that). On the lingual side of the denture, in the area for the female part of the locator, perforations were made in order to extract the surplus of the acrylate, so as not to remain trapped gingival. After bonding the acrylate, on the female part of the locator there are working rubbers (black). After removal of the denture from the oral cavity, the working rubber was removed by LOCATOR® Core Tool key and a 0-10 ° rubber was set, i.e. 10-20 ° depending on the need to correct the disparity. In this patient, rubbers that tolerate diparality up to 10 degrees (retention forces of 3.0 lbs) were placed (Figure 1).

**Figure 1.** Gingival display of the denture with 10 degree rubbers, retention forces 3.0 lbs

After that, the cementing of the telescope to the tooth 33 was done with all the preparations that require the cementing of the telescope system (vaseline isolation of the inner surface of the outer telescope crown and the outer surface of the inner telescope crown) (Figure 2).

**Figure 2.** Isolation with vaseline of the outer surface of the inner crown



Zinc phosphate cement (Harvard, Germany) was used for the cementing of the inner telescope crown. After cementing the telescope system, the surplus of zinc phosphate cement was removed, which was visible to the naked eye. A possible correction of the occlusion with articulation paper of 80 μ (Coltene / Whaledent) was made. On the control examination after 24 hours, the denture was removed (Figure 3, Figure 4, Figure 5). Instruction of the patient for removal and putting the denture was performed, instructions for maintenance of oral hygiene and denture were given to the patient. Also, during the examination, the reocclusion with articulation paper of 80 μm was done. At the controls of every 3 months, the presence of periimplantitis or bone resorption around the implant, as well as bone resorption around the teeth carrying the telescope crown, was not clinically and radiographically established.

**Figure 3.** Review of male parts of locator and inner conus telescop system

**Figure 4.** Definitive prosthetic restoration

**Figure 5.** Definitive prosthetic restoration - lingual aspect

## CASE REPORT 2 - THE SPECIFICITY OF THE CASE

A 55-year-old patient came to dental office for inadequate stabilization and poor retention of the lower RPD. The analysis of the anamnestic questionnaire found that the patient does not have general chronic diseases and does not use any type of therapy. Implant planning and implantation of dental implant was performed on the basis of CBCT analysis and evaluation. In the dental status in the lower jaw on the left side there was an intact first premolar. In the upper jaw there was a complete denture. After implantation of dental implant in region 33 (Implant direct legacy 3, USA), and 3-month bone oseointegration period, a prosthetic rehabilitation of the patient was done. All clinical and laboratory phases are exactly the same as in the previous case. The difference is that the disparity between the axis of the implant and the axis of the tooth is greater than 10 degrees, so the rubbers that correct the disparity of 10-20 degrees were placed. At the controls of every 3 months, the presence of periimplantitis or bone resorption around the implant, as well as bone resorption around the teeth carrying the telescope crown, was not clinically and radiographically established. Review of clinical phases (Figure 6 - 10).

**Figure 6.** Intraoral review after impression

**Figure 7**. Functional impression of lower jaw with elastomeric impression material

**Figure 8.** Gingival aspect of denture with working rubber (black)

**Figure 9.** Gingival aspect of denture with rubber that correct disparity till 20 degrees, retention forces 2,0 lbs

**Figure 10.** Lingual aspect of definitive prosthetic restoration

## CASE REPORT 3 - THE SPECIFICITY OF THE CASE

A 67-year-old patient came for inadequate stabilization and poor retention of the upper RPD. The analysis of the anamnestic questionnaire found that the patient does not have general chronic diseases and does not use any type of therapy. During intraoral clinical examination, in the upper jaw the presence of RPD with poor retention and stabilization and abraded teeth was detected, while in the lower jaw there was a complete denture on one telescope system. After talking with the patient, prosthetic treatment with implantation of dental implant at strategically important positions and making of combined supported overdenture was proposed.

Dental implants were implanted in the region of 14, 11, 21 (Straumann bone level implant, Basel, Switzerland) and 3-month bone oseointegration period, prosthetic rehabilitation of the patient was done. In the dental status in the upper jaw on the right side there is an intact second molar. All clinical stages are exactly the same as in the previous case. The difference is in the technical phase in which the female parts of the locator in the intercanine segment were coated with an opaque because the implants were embedded in the alveolus of the extracted teeth, and after the period of the oseointegration due to vestibular resorption of the bone, implants were too vestibulary (Figure 11). Also, the difference is that the rubbers on the locators in region 11, 21 were of 10-20 degrees. At the controls of every 3 months, the presence of periimplantitis or bone resorption around the implant, as well as bone resorption around the teeth carrying the telescope crown, was not clinically and radiographically established. Review of clinical phases (Figure 11-14).

**Figure11.** Intraoral finding with female parts of locator system

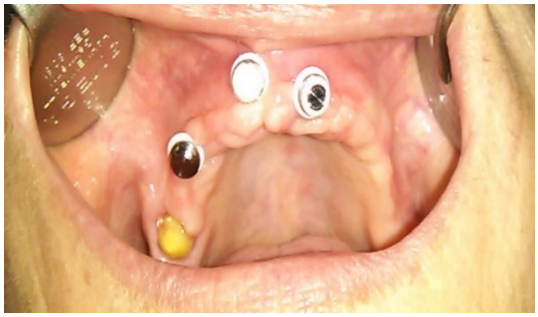


**Figure 12.** Intraoral finding after cementing of telescop system

**Figure 13.** Occlusal aspect of prosthetic restoration

**Figure 14.** Definitive prosthetic restoration

## DISCUSSION

The present case series describes prosthodontic rehabilitation of patients with double crown and locator atachments-retained overdentures supported by a combination of natural tooth and strategic implants. Namely, the rehabilitation of an partially edentulous jaw with only a few remaining teeth often requires the use of implants as supplementary abutments to increase favorable support zone (quadrangular or triangular), patient comfort and satisfaction. However, the combination of teeth and implants for the support of removable partial denture (RPD) has been rarely described in the literature (15,16,18-22). Previous studies (16,18,21,22) have reported survival and prosthetic complications for combined tooth-implant-supported overdentures on double crowns as homogenous anchorage element on teeth and implants. The results of these studies revealed that the tooth-implant supported double crown-retained overdentures had high implant survival rate of 92% to 100% after observation period of 3 to 6 years (16,18,21,22). Furthermore, a few clinical investigations (15,16,21,22) showed that combination of implants and teeth for the support of double-crowns retained-overdentures did not result in more biological and technical complications than for dentures retained exclusively on implants or natural teeth. Also, the literature reported that several factors such as the selection of remaining teeth, the total number and the strategic distribution of the abutments are very important to the long term success of these types of prosthodontic options (15,20,22).

On the other side, Chen et al. (23) in its biomechanical study focused on the biomechanical behaviors of natural teeth in combined tooth-implant double-crown-retained prostheses. They reported that the combination of teeth and implants in rigid connectors acts as a cantilever and the highest stress concentration was observed at cortical region around the implants under loading. Also, the authors showed that the treatment of combined tooth-implant supported double crown prosthesis could protect teeth and their periodontal support tissues through relieving their loading during bearing masticatory forces, acting as a rigid splint. This finding is in line with results of clinical studies (16,22,24), in which no tooth intrusion has been reported.

In the present case series, we showed prosthetic treatments of patients with combined tooth-implant-supported overdentures retained double crown and locator attachments as heteregenous connecting elements on tooth and implants. The major advantages of this treatment option are good denture's retention and stability, improved chewing performance, good esthetic outcome and low cost. Also, keeping one or a few remaining teeth provide tactility and the reflex control of the masticatory system by the periodontal mechanoreceptors. However, in the literature, we found only two studies (16,19) in which the authors used heteregenous anchorage elements on tooth and implants for combined tooth–implant-supported RPDs. Kaufmann et al. (19) investigated telescopic crowns and ball attachments as connecting elements for combined tooth–implant-supported overdenture. In this study, higher technical complication rate was observed for overdentures retained telescopic crowns and ball attachments as anchors than for dentures retained a uniform attachment system (18,21). On contrary, Hug et al. (16) using the same anchorage elements showed that technical complications and service performed were significantly higher in the tooth root group than in implant and combined tooth-implant groups. However, satisfaction, dentures's stability, comfort and speaking ability improved in implants supported RPD wearers compared with combined or exclusive root supported RPD wearers.

## CONCLUSION

The presented treatment resulted improved functionality, comfort, aesthetics and stability after poorly fitting dentures were replaced with new, better-fitting ones. The use of double crown and locator attechments on tooth and implants to retain overdentures is reliable treatment option and can be integrated into treatment planning for patients with a highly reduced dentition. Further prospective studies on more patients are needed to confirm the results presented in this case series and to compare this treatment option with other treatment concepts.

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# CONGENITAL ABSENCE OF SKIN ON THE RIGHT LEG AND NAIL ABNORMALITIES-EPIDERMOLYSIS BULLOSA OR BART'S SYNDROM ?

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# UROĐENI NEDOSTATAK KOŽE NA DESNOJ NOZI I ABNORMALNOST NOKATNE PLOČE - BULOZNA EPIDERMOLIZA ILI BARTOV SINDROM?

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## ABSTRACT

*Children born with the epidermolysis bullosa (so-called "butterfly children") can eat only liquid or soft food due to the blisters on their mouth, tongue and esophagus. Due to their inactivity and permanent wounds, their fingers are curved and grown with a fist. Their eyes, anus and genitals are not spared either. The digestion is usually poor, so they often suffer from the constipation, and sometimes the intestine discharge can be performed only surgically. Due to frequent and numerous wounds, infections may develop, which can lead to sepsis. Wounds are caused by any kind of the pressure and re-bandaging of wounds is the most painful. These children can later be susceptible to other diseases, especially the skin cancer. More than 80% of children diagnosed with this disease become disabled in the first years of their lives, and some of them pass away immediately after birth. The average lifespan of the diseased is about 28 years. Here we have presented a rare case of a newborn male infant with a dystrophic epidermolysis bullousa, a congenital skin aplasia on the right leg and a nail dystrophy. Based on a typical clinical presentation, we think that it is Bart's syndrome.*

***Keywords**: epidermolysis bullosa, "butterfly children", Bart's syndrome*

## SAŽETAK

*Deca rođena sa buloznom epidermolizom (takozvana „deca leptiri") mogu da jedu samo tečnu ili kašastu hranu zbog plikova na ustima, jeziku i jednjaku. Zbog neaktivnosti i stalnih rana prsti im se krive i srastaju sa šakom. Nisu pošteđene ni oči, analni otvor i genitalije. Varenje je obično loše, pa često imaju opstipaciju, a ponekad se pražnjenje creva može izvršiti samo hirurški. Usled čestih i mnogobrojnih rana, dolazi do infekcija koje mogu dovesti do sepse. Rane izaziva svaka vrsta pritiska, a najbolnije je previjanje. Ova deca su kasnije podložnija drugim bolestima, a posebno karcinomu kože. Preko 80% obolelih postaju invalidi već u prvim godinama života, a neki od njih umiru neposredno po rođenju. Prosečan životni vek obolelih je 28 godina. U ovom radu smo predstavili redak slučaj muškog novorođenčeta sa distrofičnom bulloznom epidermolizom, urođenom aplazijom kože na desnoj nozi i distrofijom nokta. Na osnovu tipične kliničke prezentacije mislimo da je u pitanju Bartov sindrom.*

**Ključne reči:** *bulozna epidermoliza, „deca leptiri", Bartov sindrom*

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## INTRODUCTION

Epidermolysis bullosa (EB) is an inherited mechanobullous disorder characterized by skin fragility and blister formation, following minor trauma or traction on the skin. Children born with the EB are commonly referred to as "butterfly children" due to their skin being so fragile it mimics a butterfly wing. The EB encompasses many clinically distinctive phenotypes: the EB simplex (EBS), the junctional EB (JEB), the dystrophic EB (DEB) and the Kindler syndrome (KS). The EBS was further separated into suprabasal and basal subgroups, based on the histopathologic site of cleavage within the epidermis. (1, 2)

As a correlate, the presence of diagnostically useful skin findings (e.g. exuberant granulation tissue, mottled pigmentation, pseudosyndactyly and other) may permit further subclassification at this level. Each patient then can be further subclassified on the basis of a mode of transmission and, if identifiable, by the specific gene involved, the latter initially determined by means of immunohistochemical techniques (IFM, using EB-pertinent monoclonal antibodies) and later by the mutation analysis. In some clinical settings, some EB investigators prefer to pursue molecular screening without first obtaining of IFM results. The EB can be inherited in the dominant or recessive form, it can also arise as a spontaneous mutation where neither parent carries EB or is physically affected by the condition. The gene mutation occurs spontaneously in the sperm or egg before conception. (1, 3)

The overall prevalence of EB has been estimated to be about 1 in 100,000 in Italy, 1 in 130,000 in the United States, 1 in 20,000 in Scotland and in Croatia it is 9.6 cases per million live births. The number of the diseased in Serbia is still unknown, but according to the latest data there are about 140. The incidence and prevalence of dominant DEB were found to be 2.12 and 1.49 cases per 1 million live births and recessive DEB were found to be 3.05 and 1.35 cases per 1 million live births. (4, 5)

In our case, the clinical finding of DEB, the Aplasio Cutis Congenital (ACC) and nail dystrophy shows that it is Bart's syndrome, first described by Bruce J. Bart in 1966. Bart's syndrome is usually diagnosed based on the clinical presentation. In some cases, the analysis may require skin biopsy to determine the type of epidermolysis bullosa and genetic study to look for the exact gene mutation that may help to confirm the final diagnosis. (6)

## CASE REPORT

A term newborn male delivered by Caesarean section (C-section) after an uncomplicated pregnancy and spontaneous rupture of membranes approximately 32 hours prior to delivery. Apgar score was 8 at 5 minutes. The baby's weight was 2780 g and the head circumference was 33 cm. Upon initial physical examination, the patient was presented with multiple skin defects: on the anterior side of the lower third of the upper leg, knee, lower leg and whole feet, on the dorsal parts of the fist and fingers, on the head skin frontal and temporal right, in the area of the upper lip and right hemithorax. (Picture1.)

**Picture 1.** Appearance of the newborn after birth

These defects were dark red color, dry and slightly deeper on the right leg with visible vascularization. Also, there were rarely collapsing blisters. On the front of the right leg, including the knee, foot dorsum, thumb and right-foot sole, the complete absence of the skin, peripheral with the individual haemorrhagic blisters. The nail plate on the right foot is missing. Haemorrhagic blisters were present on some finger nails and toes of the fist and feet. (Picture 2.)

**Picture 2.** Congenital localized absence of skin, blistering of the skin and absence of nail plate on the right foot

Other physical findings were normal for the age. Baby was transferred to the Neonatal Intensive Care Unit (NICU), on the first day of life.

In the NICU, the intravenous catheter for rehydratation was placed to prevent dehydration from extended evaporation from the skin and for initial dual antibiotic therapy (cefotaxime - Tolycar® and vancomycin) to prevent infection. The inflammation parameters were elevated, the C reactive protein was 23 mg/L, while procalcitonin was 4.450 ng/ml. Blood and urine results were normal for the age. Abdominal and cranial ultrasound imaging, and echocardiography revealed normal findings. Bacteriological analysis of eye swabs, nasal swabs and erosion swabs (axillary region, knees, fists, right upper arm and knee) have shown normal flora. He received phenobarbitone due to sedation and acetaminofene (Paracetamol®) as an analgesic. The skin was treated according to a Dermatologist's advice (bandage once a day, 1% chloramphenicol unguent daily on skin defects). After four days, the patient was transferred to the Clinical Center of Serbia (CCS), Pediatric department, for further treatment and examination. A Dermatologist's physical examination in the CCS has also identified a variety of skin changes and congenital aplasia of the skin on the right leg and foot and nail dystrophy.

Topical treatment for all erosion was 0,1% gentamicin unguent, and than wrapping with sterile gauze (Mepilex® Transfer gauze) and bandaging soaked with vaseline once a day and for eyes 1% chloramphenicol unguent daily. Skin zones with residual changes and without erosion were treated with emollients once a day. For erythema on the face 1% hydrocortison unguent was used twice per day and for erythema in the diaper region miconazole (Dactanol®) gel several times per day. Regarding skin care and daily bathing, oil bath (Lipikar syndet baby®) and nonadhesive re-dressing were prescribed. The substitution of vitamin D 500 IU./mL was recommended once per day. After 3 weeks, the erosion was in the final phase of epithelialization. The zone of congenital skin absence on the right lower leg was in the final phase of epithelialization. The erythema on the face was significantly bleached. (Picture 3.) The general condition was well and the mother was given detailed instructions about handling the baby and continuing with the local wound care. Further therapy was performed by 0,1% gentamicin unguent and emollients and bandage Mepilex® Transfer gauze and separation of fingers by Mepilex®.

In the fourth month of life the child was diagnosed with Dermatitis atopica, the child has the highest number of erosions on the face and ear shells with eczema and itching. After 1% hydrocortison unguent therapy for 7 days, the child had an erythema with white squamous on cheeks. Levocetirizine (Xyzal®) 0,5 mg/ml oral solution was recommended twice a day for 2.5 ml in the case of itching and eczema exacerbation.

**Picture 3.** After topical treatment in the final phase of epithelialization

## DISCUSSION

The EB is a very rare disease, especially in the form of congenital skin aplasia. In previous studies, it has been shown that the EB manifestation is not only limited to the skin. Systemic signs might involve the nose, ear, eye, genitourinary tract and upper gastrointestinal tract. A. Michalak et al. have described in their work that gastrointestinal manifestation of the EB is most commonly reflected by esophageal stenosis due to recurrent esophageal blistering, followed by consequent scarring. (7) Also, in study of nine cases with the Congenital Pyloric Atresia (CPA), M.Kansra et al. had one patient with the CPA (type 3) and the ileal atresia associated with the EB. (8) In our case the EB is in combination with congenital skin aplasia, which is based on the author Frieden, in 6th group and it is called Bart's syndrome. The inheritance pattern of Bart's syndrome appears to be an autosomal dominant. Nevertheless, several sporadic cases have been reported. Bart's syndrome is considered as an exceedingly rare genetic disorder. (9) Our case of Bart's syndrome presented with the classic triad of congenital localized absence of skin over right lower leg, blistering of the skin, and absence of nail plate on the right foot.

When evaluating newborns with plaques and/or erosion, in addition to numerous diagnostic conditions, the EB should be taken into consideration. The differential diagnosis for blisters in a neonate is extensive and includes common acquired etiologies such as sucking blisters or other birth trauma-induced blisters, infection-related blisters such as the herpes simplex, the bullous impetigo, the staphylococcal scalded skin syndrome, the neonatal candidiasis, the neonatal varicella; maternal autoimmune bullous conditions such as the bullous pemphigoid (can also appear de novo), the pemphigoid gestationis or the pemphigus vulgaris; others such as a bullous aplasia cutis congenita, and bullous mastocytosis; and genetic disorders including the incontinentia pigmenti, the ectodermal dysplasia, the epidermolytic hyperkeratosis (bullous congenital ichtyosiform erythroderma), the pachyonychia congenita, the congenital erosive dermatosis with

reticulated supple scarring and the epidermolysis bullosa (all subtypes). (2) Analogous to peeling an onion, Jo-David Fine et al. suggested that the classification and subclassification of patients with the EB begin with their separation into 1 of 4 main EB groups, based on the level (intraepidermal [EBS]; within [JEB] or beneath [DEB] the skin basement membrane zone (BMZ); or mixed pattern [Kindler syndrome]) within which blisters develop. In EB simplex (EBS) suprabasal, the blisters form within the middle/upper epidermal layers, depending on which protein is mutated. In the EBS basal, the cleavage plain is within the basal keratinocytes. In junctional EB (JEB), the separation takes place within the lamina lucida, and in dystrophic EB (DEB), within the sublamina densa region within the uppermost dermis. In the fourth type cleavage it can occur within the basal keratinocytes, at the level of the lamina lucida or below the lamina densa and it is Kindler syndrome [KS], mixed pattern. Further, intraepidermal EBS is divided into two groups: suprabasal and basal EBS. Targeted proteins by the gene mutation in suprabasal EBS are Transglutaminase 5 (TGM5), plakophilin 1 (JUP), desmoplakin (DSP) and plakoglobin (PKP1); in basal EBS are keratins 5 or 14 (KRT5, KRT14), plectin (PLEC), exophilin 5 Slac2-b (EXPH5) and bullous pemphigoid antigen 1 (DST). Changes in JEB are in intralamina lucida and in the generalized form affected proteins are: laminin-332 (LAMA3A), collagen XVII (COL17A1), α6β4 integrin (ITGB4, TGA6), α3 integrin subunit; in localized form: collagen XVII, laminin-332, α6β4 integrin. In DEB only collagen VII (COL7A1) is affected. (1, 10) The next level of subclassification takes into account the clinical phenotypic features present in a given patient, most notably the distribution (localized vs generalized) and the relative severity of cutaneous and extracutaneous disease involvement. Ensuring that the correct treatment plan and dressing regimen are implemented with EB patients is challenging due to painful open wounds and the exceptionally fragile skin.

## CONCLUSION

Epidermolysis bullosa is a rare, hereditary, non-contagious, but still incurable disease. The average lifespan of the diseased is shortened, requiring constant care and complete dependence on other people.

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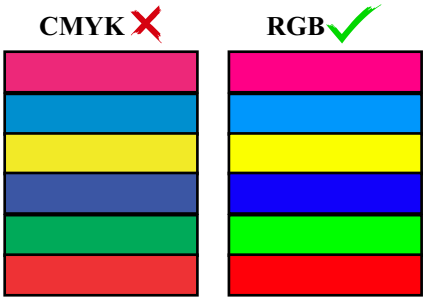


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